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EXAMPLE 32

CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

5 A 2H7 scFv IgG fusion proteins are constructed with the first cysteine residue and the second cystein in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC). The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO:___). The oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HIgGMHcys1 (SEQ ID NO:___) and HIgGMHcys2 (SEQ ID NO:___). The constructs are prepared as described in SEQ ID NO:___). The encoding polynucleotides of the mutants are presented in SEQ ID NOs:___) and the polypeptide sequences are provided in SEQ ID NO:___).

Additional representative sequences of the present invention are as follows:

15

HuIgG1 wild type hinge, CH2, CH3

tctgatcaggagcccaaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtcttctctt
ccccccaaaaccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtgggacgtgagccacgaagaccctgag
20 gtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg
tggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccc
atcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatccgggatgagctgaccaaga
accaggtcagcctgacctgcctgtgcaaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaaca
ctacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggg
25 gaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

HuIgG1 wild type hinge, CH2, CH3

sdqepkscdkthtcppcpapellggpsvflfpkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvev
hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietkiskakgqprepqvylppsrdeitknqvsitclvkgyf
30 psdiavewesngqpennykttppvlidsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

Llama IgG1 hinge, CH2, CH3

tgalcaagaaccacatggaggatgcacgtgcccnacgtgcccncaatgccnngcncnngaactnccaggaggc
ccttctgtctttgtcttcccccgaaacccaaggacgtcctctccatttttgaggccgagtcacgtgcgttgtagtgagcgtcggaagaaa

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gaccccgaggtcaatttcaactggatatattgatggcgttgaggtgcgaacggccaafacgaagccaaaagaggaacagttcaacagcacg
taccgcgtggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgcagggtcaacaacaagctctccc
ggccccatcgagaggaccatctccaaggccaaagggcagaccgggagccgcaggtgtacacctggccccacaccgggaagaac
tgccaaggacaccgtgagcgttaacatgcctgggtcaaaggctctacccagctgacatcaacgttgagtggcagaggaacggtcagccg
5 gagtgcagagggcacctacgccaacacgccccacagctggacaacgacgggacctaactctctacagcaagctctcgggtgggaaga
acacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgagggcctgcacaaccactacaccagaaatccatcaccagctctt
cgggtaaataagtaatactaga

Llama IgG1 hinge, CH2, CH3 (In figure 23 as Llama IgG1)

10 ephggctcpqcpapelpggpsvfvfpkpkdvlslsgrpevtcvvvdvgkedpevnfnwyidgvevrtantk
pkeeqfnstyrvvsvlpiqhqdwtgkefkckvnnkalpapiertiskakgqtrepqvytlaphreelakdtvsvtclvkgfypadin
ewqrngqpeseqgyantppqlndgtyflysrlsvgkntwqrgetltgvvmhealhnhytqksitqssgk

Llama IgG2:

15 tgatcaagaacccaagacacaaaaaccacaaccacaaccacaaccacaaccacaaccatctacaacagaatccaagtgtcccaatgtccag
cccctgagctctctgggagggccctcagttctatcttcccccgaaacccaaggacgtcctctccattctctgggagggccgaggtcacgtg
cggtgtggtagacgtgggccaggaagaccccgaggtcagttcaactgggtacattgatggcgtgaggtgcgaacggccaacacgagggc
caaaagagggaacagttcaacagcacgtaccgctgggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaa
gtgcaaggtcaacaacaagctctccccggccccatcgagaagaccatctccaaggccaaagggcagaccgggagccgcaggtgta
20 caccctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacatgcctgggtcaaaggcttctacccacctgatatcaacg
ttgagtggcagaggaatgggcagccggagtcagagggcacgtacgccaccacgccacccagctggacaacgacgggacctaactctct
ctacagcaagctctcgggtggaaagaacacgtggcagcagggagaaaccttcacctgtgtggtgatgcacgagggccctgcacaaccact
acaccagaaatccatcaccagctctcgggtaaataagtaatactaga

25 *Llama IgG2*

Dqepktpkpqpqpqpnpptteskcpkcpapellggpsvfvfpkpkdvlslsgrpevtcvvvdvgqedpev
sfnyidgaevrtantrpkeeqfnstyrvvsvlpiqhqdwtgkefkckvnnkalpapiektiskakgqtrepqvytlaphreelakdt
vsvtclvkgfypdinveqwrngqpeseqgyatppqlndgtyflysklsvgkntwqqgetftcvvmhealhnhytqksitqssgk

30 *Llama IgG3 Fc*

tgatcaagcgcaccacagcgaagacccagctccaagtgtcccaatgccaggccctgaactccttgaggggcc
cacggtctctatcttcccccgaaagccaaggacgtcctctccatcaccggaaaacctgaggtcacgtgcttgggtggagctgggtaaag
aagacctgagatcgagttcaagctggctcggatgacacagagggtacacacggctgagacaaagccaaaggaggaacagttcaaca

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gcacgtaccgcgtggtcagcgtcctgcccacccagcaccaggactggctgacggggaagggaattcaagtgaagggtcaacaacaagct
ctcccagcccccatcgaggagaccatctccaaggccaaagggcagacccgggagccgcaggtgtacacctggccccacaccgggaa
gagctggccaaggacaccgtgagcgtaacctgcctggtaaaaggcttctccagctgacatcaacgttgagtgagcagaggaatgggca
gccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttctctacagaaactctccgtggga
5 aagaacacgtggcagcaggagagaagtcttcacctgtgtggtgatgcagaggctctacacaatcactccaccagaaatccatcaccag
tcttcgggtaaatagtaatctagaggggccc

Llama IgG3 Fc

dqahhsedpsskcpkcpgpellggptvfifppkakdvlsitrkpevtclwwtwvkktrsssswsvddtevh
10 etkpkeeqfnstyrvvslpiqhqdwlgtkefkckvnnkalpapiertiskakgqtrepqvytlaphreelakdtvsvtclvkgtffpadi
nvwqmrngqpesegtyantppqlndgtyflysklsvgkntwqqgevfccvmhealnhstqlksitqssgk

15

HuIgG1 wild type hinge

gatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgccagca

HuIgG1 wild type hinge

dqepksedkthtppcpa

20

HuIgG1 H2, wild type hinge with leu at second position (results from BglI site)

gatctggagcccaaatctgtgacaaaactcacacatgccaccgtgccagca

HuIgG1 H2, wild type hinge with leu at second position.

25

dlepksedkthtppcpa

NT

HuIgG1 wild type CH2

cctgaactcctgggggaccgtcagtccttctctcccccaaaacccaaggacaccctcatgatctccggacccc
30 tgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatg
ccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg
caaggagtacaagtgcaggtctccaacaaagccctccagcccccatcgagaaaaccatctccaagccaaa

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HuIgG1 wild type CH2 AA

pellggpsvflfpkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvs
vltvlhqdwlngkeykckvsnkalpapiektiskak

5

NT HuIgG1 wild type CH3

gggcagccccgagaaccacaggtgtacacctgcccccatccgggaggagatgaccaagaaccaggtcagcc
tgacctgcctgggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccac
gcctcccgtgtggactccgacggctccttctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttca
10 tgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA HuIgG1 wild type CH3

gqprepvytlppsreemtknqvslclvkgfypsdiavewesngqpennykttppvlstdgsfflyskltvdk
15 srwqqgnvfscsvmhcalhnhytqkslspsgk

NT HuIgG1 mutated hinge (C-C-C→S-S-S)

gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca
20

AA HuIgG1 mutated hinge (C-C-C→S-S-S)

dqepkssdkthtsppspa

25 Mutant hinge, but wild type CH2 and CH3—reads from the hinge+Ig tail,
HIgG1MTH WTCH2CH3:

tgatcaccccaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagt
cttcctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtggagctgagccacgaaga
ccctgagggtcaagttcaactgggtacgtggacggcggtggaggtgcataatgccagacaaagccgaggaggagcagtacaacagcacg
30 taccgtgtgggtcagcgtctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaacaaagccctcca
gccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctga
ccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgga
gaacaactacaagaccacgcctcccggtgtggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca

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gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgat
aatctaga

Protein sequence: Mutant hinge, but wild type CH2 and CH3

5 dhpksdkthtsppssapellggpsvflfppkpkdtlmsrtpevtcvvdvshedpevkfnwyvdgvevhna
ktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltclykgfyps
iavewesngqpennyktpvldsdgsfflyskltvdkrsrwqqgnvfscsvmhealhnhytqkslspsgk

LLG1-5'bgl 35 mer Llama IgG1 5'
10 5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'

LLG2-5'bgl 32 mer, Llama IgG2-5'
5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

15 LLG3-5'bgl 33 mer, Llama IgG3-5'
5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

LLseqsense 19mer, llama sequencing primer
20 5'-ctg aga tcg agt tca gct g-3'

LLseqAS 19 mer
5'-cct cct ttg gct ttg tct c-3'

NT
25 **2H7 scFv llama IgG1**

aagcttgccgcatggattttcaagtgacagatttcagcttctgctaatacagtgcttcagtcataattgccagaggaca
aattgttctctccagctcagcaatcctgtctgcatctccaggggagaaggcacaatgacttgaggccagctcaagtgttaagtacat
gcactgggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
gcagtgggtctgggaccttactctctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgagggtttaacc
30 caccacgttcgggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggatctggaggaggtgggagctctca
ggcttatctacagcagcttggggctgagctggtagggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacattaccagtt
acaatatgcactgggtaaagcagacacctagacaggcctggaatggagctatttatccaggaaatggatgatacttctacaatcag
aagttcaaggggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg

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gtctatttctgtgcaagagtgggtactatagtaactcttactggacttcgatgtctggggcacagggaccacggtcaccgtctcttgatca
agaaccacatggaggatgcacgtgccncagtgccncaatgccnngcncngaactnccaggaggcccttctgtcttcttcccccc
gaaaccaaggacgtcctctccattttggaggccgagtcacgtgcgttgtagtgagcgtcggaagaaagaccccagggtcaattcaac
tggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagggaacagttcaacagcacgtaccgcgtggtcagcgtcctg
5 cccatccagcaccaggactggctgacggggaaggaattcaagtgaaggtaacaacaagctctccggcccccacagaggacca
tctccaaggccaaaggcgagaccgggagccgaggtgtacacctggccccacaccgggaagaactggccaaggacaccgtgagc
gtaacatgcctggtcaaaggcttctaccagctgacatcaacgttgagtgagcagaggaaacggtcagccggagtcagagggcacctacgc
caacacgccccacagctggacaacgacgggacactctctctacagcaagctctcgggtgggaaagaacacgtggcagcggggaga
aaccttaacctgtgtggtgatgcatgaggccctgcacaaccactacaccagaaatccatcaccagctctcgggtaaatagtaactaga
10

AA 2H7 scFv llama IgG1

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsn
lasgvparfsgsgsytstisrveadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
15 mskcasgyttsynmhwwkqtrpqglewigaiypngdtsynqkfkgtatltvdkssstaymqlssltedsavyfcarvvyysns
ywyfdvwtgtttvtssdqephggctcpqcpapelpggsvfvfpkpkdvlsifggrvtcvvvdvgkkdpevnfnwyidggev
rtantkpeeqfnstyrvsvlpiqhqdwtgkefkckvnnkalpapiertiskakgtrepqvylaphreelakdivsvtclvkgyfyp
adinvewqrngqpeseqgyantppqlndgtyflysklsvgkntwqrgetltcvvmhealnhhtyqksitqssgk
20

NT 2H7 scFv llama IgG2

aagcttgccgccatggatttcaagtgcagatttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca
aattgttctctccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacat
gcactgggtaccagcagaagccaggatcctcccccacccctggatttatgcccatccaacctggcttctggagtcctgtctgccttcagtg
25 gcagtggtgtctggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggttttaacc
caccacgttcgggtgtgggaccaagctggagctgaagatggcggtggctcggcggtggtggatctggaggaggtgggagctctca
ggcttatctacagcagcttggggctgagctggtagggcctggggcctcagtgagatgtcctgcaaggcttctggctacacattaccagtt
acaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctattatccaggaaatggtgatacttctacaatcag
aagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
30 gtctatttctgtgcaagagtgggtactatagtaactcttactggacttcgatgtctggggcacagggaccacggtcaccgtctcttgatca
agaaccaagacacaaaaaccacaaccacaaccacaaccacaaccacaaccatctacaagaatccaagtgcccaaatgtccagcccctg
agctcctgggaggggccctcagcttctctctccccgaaaccaaggacgtcctctccatttctgggaggcccagggtcacgtgcgttg
gtagacgtgggcccaggaagaccccagggtcagttcaactgggtacattgatggcgtgaggtgcgaacggccaacacaggccaaaag

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aggaacagttaacagcacgtaccgcgtggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgc
ggtaacaacaaagctctccggcccccacgagaagaccatctccaaggccaaaggcgagaccgggagccgcaggtgtacacct
ggccccacaccgggaagagctggccaaggacaccgtgagcgtaacatgcctggtaaaaggcttciaccacctgatatcaacgttgagt
ggcagaggaatgggcagccggagtcagagggcacgtacgccaccacgccacccagctggacaacgacgggacctaactctctaca
5 gcaagctctcggtaggaagaacacgtggcagcaggagaaaccttcacctgtgtggtgatgcagaggccctgcacaaccactacacc
cagaatccatcacccagctctcgggtaaatagtaattctaga

AA

2H7 scFv llama IgG2

0 mdfqvqifsfllisaviiargqivlsqpailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypsn
lasgvparifsgsgtsysltisrveadaatyycqwsfnptfagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytfsynmhwwkqtpqrqglewigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavyfcarvvyysns
ywyfdvwgtgtvtvssdqepktpkpqpqpqpnppteskepckpapellggpsvfifppkpkdvlisgrpevtcvvvdvqged
pevsfnwyidgaevrtantrpkeeqfnstyrvvsvlpiqhqdwtgkefkckvnnkalpapietiskakgtrepqvytlaphreela
15 kdtvsvtclvkgyfppdinveqmrngqpesegtyatppqlndngtyflysklsvgkntwqqgetftcvvmhealhnhytqksitqss
gk

NT

2H7 scFv llama IgG3

20 aagcttgccgcatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca
aattgttctctccagctctcagcaatcctgtctgcattctccagggagaaaggtcacatgacttcagggccagctcaagtgtaatgacat
gcactgggtaccagcagaagccagatcctccccaaacctggattatgccccatccaacctggcttctggagtcctgctcgttcagtg
gcagtgggtctgggacctcttactctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgaggatttaacc
caccacgftcgggtgctgggaccaagctggagctgaagatggcggtggtcggcggtggtggatctggaggagtgaggctctca
25 ggcttatctacagcagctctggggctgagctggtgagcctggggcctcagtgaaagatgctcgaaggcttctggctacacattaccagtt
acaatatgcactgggtaagcagacacntagacaggcctggaatggattggagctatttatccaggaaatggtgatacttctcacaatcag
aagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
gtctatttctgtgcaagagtgggtactatagtaactcttactggtactcgtgctggggcacagggaaccggcaccgtctctctgatca
agcgaaccacagcgaagacccagctccaagtgtcccaaatgccaggccctgaactcctggaggggccacggctctcatcttcccccc
30 gaaagccaaggacgtcctctccatcacccgaaaacctgaggtcagctgctgtggtgacgtgggtaaagaagacctgagatcaggttc
aagctggctcgtgatgacacagaggtacacacggctgagacaaagccaaggaggaacagttaacagcacgtaccgcgtggtcagc
gtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgaaggtcaacaacaaagctctccagcccccatcgagag
gaccatctcaaggccaagggcagacccgggagccgaggtgtacacctggccccacaccgggaagagctggccaaggacaccg

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tgagc gtaacctgcctgggtcaaaggcttctccagctgacatcaacgttgagtgagcagaggatgggcagccggagtcagagggcacct
acgccaacacgccgccacagctggacaacacgggacctaacttctctacagcaactctcgtgggaaagaacacgtggcagcaggg
agaagtcttcacctgtgtggtgatgcacgaggtctacacaatactccaccagaaatccatcaccagctctcgggtaaatagtaatactag
agggc

5

AA

2H7 scFv llama IgG3

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsn
lasgvparfsgsgsgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytftsnnmhvwkqtrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavvyfcarvvyysns
ywyfdvwgtgtvtvssdqahshsedpsskcpkcpellggptvffppkakdvlsitrkpevtclwwtwvktlrsssswsvddt
evhtaetkpkkeeqfnstyrvvslpiqhqdwtgkefkckvnnkalpapiertiskakgqtreppvytlaphreelakdtsvtclvkf
fpadinvwqrngqpeseqtyantppqlndngtyflysklsvgkntwqqgevtcvvmhealnhstqksitqssgk

15

2H7+Completely WT IgG tail:

2H7 scFv WTH WTCH2CH3

Nucleotide sequence:

aagcttgccgcatggatttcaagtgcagattttcagcttctgtaatactgcttcagtcataattgccagaggaca
aattgttctctccagctcagcaatctgtctgcatctccagggagaaagtcacaatgacttcagggccagctcaagtgttaattacat
gcactgggtaccagcagaagccaggaatctccccaaacctggatttatgcccatccaacctggcttctggagtcctctgctcgttcagt
gcagtgggtctgggaccttctactctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgagggtttaacc
caccacagcttgggtgctgggaccaagctggagctgaagatggcggtgctcggcggtggtgagtcggaggagggtgggagctctca
ggcttatctacagcagcttgggctgagctggtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacattaccagtt
acaatatgcactgggttaaagcagacacctagacaggcctggaatggattggagctattatccaggaaatgggtatacttctacaatcag
aagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
gtctatttctgtgcaagagtggtgtactatagtaacttctactgttactcgtctggtgggcacagggaccacgggtcaccgtctctctgatca
ggagcccaaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagcttctcttccccca
aaaccCaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggagctgagccagaagacctgaggtcaagtt
caactgggtacgtggagcgcgtggaggtgataatgccaagacaaagccgaggaggagcagtaaacagcacgtaccgtgtggtcagc
gtctcaccgtctgcaccagactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagccccatcgagaa
aacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatcccggtgagctgaccaagaaccaggtc
agcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagacaactacaaga

30

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ccacgcctcccggtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccggtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7+Completely WT IgG tail:

5

2H7 scFv WTH WTCH2CH3

Protein sequence

mdfqvqifsfllisasviiargqivlsqpailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiypsn
lasgvparfsgsgsgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytftsymbhwwkqprqglewigaiypngdtsynqkfkkgkatltvdkssstaymqllsitsedsavvyfcarvvyysns
10 ywyfdvwtgtttvssdqepkscdkthtppcpapelggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietkiskakgqprepvytlppsrdeitknqvslclvk
gfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispk

NT

15

CD80 transmembrane domain and cytoplasmic tail (+restriction sites)

goggatcctcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacc
tactgctttgccccaatgagcagagagagaaggaggaatgagagattgagaagggaagtgtacgcctgtataaatcgat

AA

20

CD80 transmembrane domain and cytoplasmic tail

adpsnllpswaitlisvngifviccltycfaprerrrrmerlrresvrpv

NT

25

40.2.220 VL (anti-human CD40 scFv #1--VL)

aagcttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacatt
gttctgactcagctccagccaccctgtctgtgactccaggagatagagctctcttctcagggccagccagagtattagcgactacttac
actggtatcaacaaaatcacatgagctccaaggcttctcatcaaatatgcttccattccatctctgggatccctccaggttcagtggcagt
ggatcagggtcagatttactctcagatcaacagtggtgaacctgaagatgttggaattattactgtcaacatggtcacagctttccgtggac
30 gttcgggtggaggcaccaagctggaaatcaaacgg

AA

40.2.220 VL (anti-human CD40 scFv #1--VL)

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mdfqvqifsfllisasvimsrgvdivltqspatlsvtpgdrvslsruasqsisdylhwyqqkshesprllikyashsi
sgipsrfsfgsgsgsdftlsinsvepedvgiyycqhgshfpwtfgggtkleikr

NT

5 40.2.220 VH (for anti-human CD40 scFv #1--VH)

cagatccagttgggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctg
ggatgccttcacaactactggaatgcagtgagggtgcaagagatgccaggaaagggttgagtgaggatggctggataaacacccactctg
gagtgccaaaatatgtagaagacttcaaggacgggttgccttctctttggaacctctgccaactgcataattacagataagcaacctcaaa
gatgaggacacggctacgtatttctgtgtgagatccgggaatggaactatgacctggcctactttgcttactggggccaaggacactggg
10 cactgtctctgatca

AA

40.2.220 VH (for anti-human CD40 scFv #1--VH)
qiqlvqsgpelkkpgetvriskasgyafttgmqvwqempgkglkwigwintplwsakicrrlqgrfafslets
15 antaylqisnlkdedtatyfcvrsngnydlayfaywgqgtlvts

NT

40.2.220 scFv (anti-human CD40 scFv #1)
20 aagcttatggattttcaagtcagatcttctcagcttctcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatt
gttctgactcagtcctcagccaccctgtctgtgactccaggagatagagctctcttctcctgaggccagccagagattatgcgactacttac
actggatcaacaaaaatcacatgagtcctcaaggcttctcatcaaatatgcttccattccatctctgggatccctccaggttcagtggcagt
ggatcagggtcagatttactctcagatcaaacagtggtggaacctgaagatgttggaatttactgtcaacatggtcacagctttccgtggac
gttcggtggaggcaccaagctggaaatcaaacgggggtggcggtggctcgggagggtgggtcgggtggcggggatctcagatcca
25 gttgggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttcacaactactgga
atgcagtgagggtgcaagagatgccaggaaagggttgagtgaggatggctggataaacacccactctggagtgccaaaatatgtagaaga
cttcaaggacgggttgccttctctttggaacctctgccaactgcataattacagataagcaacctcaaagatgaggacacggctacgtatt
tctgtgtgagatccgggaatggaactatgacctggcctactttgcttactggggccaaggacactgggtcactgtctctgatca

AA

30 40.2.220 scFv (anti-human CD40 scFv #1

mdfqvqifsfllisasvimsrgvdivltqspatlsvtpgdrvslsruasqsisdylhwyqqkshesprllikyashsi
sgipsrfsfgsgsgsdftlsinsvepedvgiyycqhgshfpwtfgggtkleikrgggsgggsgggsgggsggiqlvqsgpelkkpgetvrisk

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ckasgyafttgmqwvqempgkglkwigwintplwsakicrrlqgrfafsletsantaylqisnlkdedtatyfcvrsngnydlayfa
ywgqgtlvtvs

NT

5 2e12 VL (with L6 VK leader peptide)

atggattttcaagtcagattttcagcttcctgctaatacagtcctcagtcataatgtccagaggagtcgacattgtgctc
acccaatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagttt
aatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctgggggtccctgccaggttt
agtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagtaggaa
10 ggttccttgagcgttcggtggaggcaccaagctggaaatcaaacgg

AA

2e12 VL (with L6 VK leader peptide)

mdfqvqifslisavimsrgvdivltqspaslavlqqratiscrasesveyyvtslmqwyqqkpgppkllis
15 aasnvsgvparfsgsgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikr

NT

2e12 VH (no leader peptide)

caggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgcaccgtctca
20 gggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtgatggaa
gcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctfaaaaatgaacagtctgcaa
actgatgacacagccagatactactgtgccagagatgggtatagtaactttcattactatgttatggactactggggtaaggaacctcagtc
ccgtctcctca(gatctg)

25 AA

2e12 VH

qvqlkesgpglvapsqslsitctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdns
ksqvflkmnslqtdtdaryycardgysnfhyvmdywgqgtsvtvss

30 NT

2e12scFv(+Restriction sites)

aagcttatggattttcaagtcagattttcagcttcctgctaatacagtcctcagtcataatgtccagaggagtcgacatt
gtgctcacccaatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtca

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caagttaatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgc
caggtttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgcagcaaag
taggaagggttccttgacgttcggtggaggcaccagctggaaatcaaacgggggtggcgggtggctcgggcggagggtgggtcgggtggc
ggcgggatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgcctcacagagcctgtccatcacatgcaccgtctcagggttc
5 tcaftaacggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtgatggaagcaca
gactataattcagctctcaatccagactgagcatcaccaaggacaactccaagagccaagtcttcttaaaaatgaacagtctgcaactgat
gacacagccagatactgtgccagagatggttatagtaactttcattatgttatggactactgggggtcaaggaacctcagtcaccgtct
cctct(gatcag)

10 AA
2e12scFv
mdfqvqifsfllisasvimrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgppkllis
aasnvesgvparfsgsgsgtdfslnihpveediamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqqlkespgplva
psqslsitctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgys
15 nfhyvymdywgqgtsvtvss

10A8 is anti-CD152 (CTLA-4)

10A8 VL (with L6 VK leader peptide)

atggattttcaagtgcagattttcagcttctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatg
20 acacagctctccatcctcactgtctgcatctctgggaggcaaaagtcacatcacttgcaaggcaagccaagacattaagaagtatataggtg
gtaccaacacaagcctggaaaagggtccaggctgctcatatattacacatctacattacagccaggcatccatcaagggttcagtggaagtg
ggctcgggagagattatccctcagcatcagaaacctggagcctgaagatttgcaactattattgtcaacagtatgataatctccattgacg
ttcggctcggggacaaagttggaaataaaacgg

25 AA
10A8 VL
mdfqvqifsfllisasvimrgvdiqmtqspsslsaslggkvtitcasqdikkyigwyqhkgpgkprlliyyst
lqpgipsrfsgsgsgrdyslsirnlepediatyycqqydnpltfsggtkleikr

30 NT
10A8 VH (no leader peptide)
gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggcta
ctccatcaccagtggtttctactggaactggatccgacagttccgggaaacaaactggaatggatgggccacataagccacgacggtagg

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aataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgag
gacacagctacatatctgtgcaagacactacggtagtagcgggagctatggactactggggtaaggaacctcagtcaccgtctcctctga
tca

5

AA

10A8 VII

dvqlqesgpglvkpsqslstcsvtgysitsgfywnwirqfpgnklewmghishdgrnnynpslinrisitrdtsk
nqfflklssvttedatatyfcarhygssgamdywgqgtsvtvss

10

NT

10A8 scFv

aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatc
cagatgacacagtcctccatcctcactgtctgcatctctgggaggcaaaagtcacatcacttgaaggcaagccaagacattaagaagtatat
aggttggtaccaacacaagcctggaaaaggtcccaggctgctcatattacacatctacattacagccaggcatcccatcaaggttcagtg
15 gaagtgggtctgggagagattatccctcagcatcagaaacctggagcctgaagataattgcaacttattattgtcaacagtatgataatctcc
attgacgttcggctcggggacaaagttggaataaaacggggtggcgggtggctcgggcgggtgggtgggtggcggcggtatctgat
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tctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgaggacacagctacatatt
20 ctgtgcaagacactacggtagtagcggagctatggactactggggtaaggaacctcagtcaccgtctcctctgatca

AA

10A8 scFv

mdfvqifsfllisasvimsrgvdiqmtqspsslsaslggkvitckasqdikkyigwyqhkpkgprlliyyst
25 lqpgipsrfsrgsgsgrdyslsirnlepediatyycqydnlpitfgsgtkleikrgggsgggsgggsgdvlqesgpglvkpsqslst
csvtgysitsgfywnwirqfpgnklewmghishdgrnnynpslinrisitrdtsknqfflklssvttedatatyfcarhygssgamdywg
qgtsvtvssd

30

NT

40.2.220-hmIgG1-hCD80

aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
gttctgactcagtcctcagccacctgtctgtgactccaggagatagagtcctctcttctgcagggccagccagagtaftagcgactacttac

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5 atgcagtggtgcaagagatgccaggaaagggtttgaagtggattggctggataaacacccactctggagtgccaaaatatgtagaaga
cttcaaggacggtttgccttctcttggaaacctctgccaacactgcataattacagataagcaacctcaaagatgaggacacggctacgtatt
tctgtgtgagatccgggaatgtaactatgacctggcctactttgcttactggggccaaggagacactggtcactgtctctgagtgaggccca
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gacacctcatgatctccggaccctgaggtcacatgcgtggtggtgacgtgagccacgaagacctgaggtcaagttcaactggtac
10 gtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctaccg
tcttcgaccaggactggtgaatggcaaggagtagaagtcaagggtctccaacaaagccctccagccccatcgagaaaacctatcc
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gcctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagacaactacaagaccacgcctcc
cgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
15 gtgatgatgaggtctgcacaaccactacacgcagaagacacctctcctgtctcgggtaagcggatccttgaacctgtcccatct
gggccattaccttaatctcagtaaatggaattttgtgatgtgctgcctgacctactgctttgccccaatgagagagagaaggaggaatg
agagattgagaagggaagtgtacgccctgtataaatcgtat

AA

20 40.2.220-hmIgG1-hCD80
mdfqvqifslisavimsrgvdivltqspatlsvtpgdrvsiscrasqsisdyllhwyqqkshesprllikyashsi
sgipsrfsrgsgsgsdftlsinsvepedvgiyyqchghsfpwtfgggtkleikrgggsgggsgggsgqqlvqsgpelkpkgetvris
ckasgyafttmqvwqempgkglkwigwintplwsakicrrlqgrfafsletsantaylqisnlkdedtatyfcvrsngnydlayfa
ywgqgtlvtvsdlepkssdkthtspspapellggssvflfppkpkdilmisrtpevtcvvdvshedpevkfnwyvdgvevhnakt
25 kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltlvkgfypsdi
vewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslspsgkadpsnllpswaitlisvn
gifviccltycfaprerrrrerlrresvrpv

NT

30 2e12scFv- hmIgG1-CD80 fusion protein
aagcttatggattttcaagtgagattttcagcttctgctaatcagtgcttcagtcataatgtccagaggagtcgacatt
gtgctcacccaatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgaattatgtca
caagtttaatgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcatacaacgtagaatctggggctcctgc

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caggtttagtggcagtggtgctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaaag
taggaagggttccttgacgttcggtggagggcaccaagctggaaatcaaacgggggtggcgggtggctcgggagggtgggtggc
ggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctcagggttc
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5 gactataatcagctctcaaatccagactgagcatcaccaggacaactccaagagccaagtttcttaaaaatgaacagctgtcgaactgat
gacacagccagatactactgtgccagagatggtfatagttaacttcattactatgttatggactactgggggtcaaggaaacctcagtcaccgtct
cctcagatctggagcccaaatctctgacaaaactcacacatccccaccgtcccagcaccigaactcctggggggatcgtcagctctcctc
ttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggagctgagccacgaagacctga
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10 gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaagggtctcaacaaagccctcccagcccc
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15 tgaacctgtcccatcctgggccattaccttaatctcagtaaatggaattttgtgatgtgctgcctgacctactgctttgccccaaagatgcaga
gagagaaggagggaatgagagattgagaagggaaggtgtacgccctgtataaatcgat

AA

2e12scFv- hmtIgG1-CD80 fusion protein

20 mdfqvqifsfllisasvimsrgvdivltqspaslavlsgqratisrasesveyyvtslmqwyqqkpgppkllis
aasnvesgvparsfsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgqvqlkesgpglva
psqslsitctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvfikmnsqtddtaryycardgys
nfhyyvmdywgqgtsvtvssdlepkssdkthtspapellggssvflfppkpkdtlmisrtpetvcvvvdvshedpevkfnwyv
dgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitknqvsitc
25 lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkadsnll
pswaitlisvngifviccltycfaprerrrrerlrresvrpv

NT

30

10A8 scFv-hmtIgG1-CD80

aagcttatggatttcaagtcagattttagcttctgctaatacagtgottcagtcataatgtccagaggagtcgacatc
cagatgacacagctctccatcctcactgtctgcatctctgggaggcaagtcaccatcacttgcaaggcaagccaagacattaagaagtatat
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cctcatgatctcccgacccttgaggtcacatgcgtgggtggacgtgagccacgaagaccctgaggtcaagttcaactgggtacgtgga
cggcgtggaggtgcataatgccaagacaaagccgcggggaggagcagtacaacagcacgtaccgtgtgtcagcgtcctcaccgtcctg
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15 ttaccttaatctcagtaaatggaattttgtgatatgctgacctactgctttgccccaaagatgcagagagagaaggaggaaatgagagatt
gagaagggaagtgtacgccctgtataaatcgat

AA

10A8 scFv-hmIgG1-CD80

20 mdfqvqifsfllisasvimsrgvdiqmtqspsslsaslggkvitckasqdikkyigwyqhkpkgprlliyyst
lqpgiprsfsgsgsgrdyslsirnlepediatyycqydnlpitfgsgtkleikrgggsgggsgggsgsdvqlqesgpglvkpsqslst
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reeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitclvkgyfypsdiave
25 wesngqpennyktpplvdsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgkadsnllpswaitlisvngif
viccltycfaprerrrrrrlresvrpv

NT

500A2-hmIgG1-CD80

30 atgtgtatacatctcagctccttgggctttactcttctggatttcagcctccagaagtacatagtgctgactcagactc
cagccactctgtcttaattcctggagaaaagtcacaatgacctgtaagaccagtcagaatattggcacaactcttactggtatcacaaa
aaccaaaggaggctccaagggtctcatcaagtatgcttcgagtcattcctgggatccctccagattcagtggcagtggttcggaaaca
gatttcactctcagcatcaataacctggagcctgatgatcggaaatttactgtcaacaaagtagaagctggcctgtcacgttcggctcctg

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gcaccaagctggagataaaacggggtggcgggtgctcggcgagggtgggtcgggtggcggcggaatctcaggtcaagctgcagcagt
ccgggtctgaactagggaacctggggcctcagtgaaactgtcctgcaagacttcaggtacataattcacagatcactatattcttgggtgaa
acagaagcctggagaaagcctgcagtggaatgaaatgttatggtgaaatgggtgtacaagctacaataaaaaatccagggaaggc
cacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgaggattctgccatctattactgtgcaagaag
5 gccggtagcgacgggcatgctatggactactggggtcaggggatccaagttaccgtctcctctgatctggagcccaatcttctgacaaa
actcacacatccccaccgtccccagcacctgaactcctgggggatcgtcagtccttcttcccccaaaaacccaaggacacctcatgat
ctcccggaacctgaggtcacatgcgtgggtgggacgtgagccacgaagacctgaggtaagttcaactggtagctggacggcgtgg
aggtgcataatgccaagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccagga
ctggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaaccatctccaaagccaaagggc
10 agccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccagggtcagcctgacctgctgggtcaaagg
cttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagacaactacaagaccacgcctcccgctgctggactccg
acggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtagcatgagggc
tctgcacaaccactacacgcagaagagcctctccctgtctcgggtaaagcggatcctcgaacctgctccatcctgggccattaccta
ctcagtaaatggaattttgtgatatgctgcctgacctactgcttggcccaagatgcagagagagaaggagggaatgagagattgagaagg
15 aaagtgtacgccctgtataaatcgat

AA

500A2-hmtIgG1-CD80

mlytsqlglfwwisarsdivltqtpatlslipgervtmtcktsqnigtilhwyhqkpkcaprailikyasqsigp
20 srfsgsgsetdftlsinnlepddigiycqqsrswpvtfgpgtkleikrgggsgggsgggsgqvkllqsgselgkpgasvklsccts
gyiftthyiswvkqkpgeslwignvygnggtsynqkfqqkatltvdkisstaymelssltedsaiyycarrpvatghamdywg
qgiqvtvssdlepkssdkthtspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktp
reeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietkiskakgqprepvytlppsrdeitknqvsitclvkgfypsdiave
wesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispgakpsnllpswaitlisvngif
25 viccltycfaprerrrrnerlrresvrpv

NT

2H7 scFv MTH(SSS)WTCH2CH3

aagcttgcgcctatggattttcaagtgagattttcagcttctcgtatcagtgcttcagtcataattgccagaggaca
30 aattgttctctccagctctcagcaatcctgtctgcatctccagggagaaagtcacaatgacttgaggccagctcaagtgtaagttacat
gcactggtaccagcagaagccaggatcctccccaacccctggatttatgccccatccaacctggctctcaggatccctgctcgttcagtg
gcagtggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggatttaaac

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cacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagctctca
ggcttatctacagcagctctggggctgagctggtgagggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacattaccagtt
acaatatgcactgggtaaacgagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatacttctacaatcag
aagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
5 gtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgactgtctggggcacagggaccacgggtcaccgtctctctgatca
ggagcccaaatcttctgacaaaactcacatccccaccgtcccagcacctgaactcctggggggaccgtcagcttctcttccccca
aancccaaggacaccctcatgatctcccgacccctgaggtcacatgcgtggtgggtgacgtgagccacgaagaccctgaggtcaagtt
caactggtacgtggacggcgtggaggtgcataatgccaagacaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagc
gtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctccagcccccatcgagaa
10 aacaatctccaaagccaaaggcagccccgagaaccacaggtgtacaccctgcccccatcccggtatgagctgaccaagaaccaggtc
agcctgacctgctgtgtaaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaaga
ccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

15 2H7 scFv MTH(SSS)WTCH2CH3 protein sequence:

mdfqvqifsfllisaviiargqivlsqspailsaspgekvmtcrasssvsymhwyqqkpgsspkpwiyps
lasgvparfsgsgsytisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytfsynmhwwkqtrqglewigaiypngdtsynqkfkkgatltvdksstaymqlssltedsavyfcarvvyysns
ywyfdvwtgtvtvssdqepkssdkthtspspapellggpsvflfpkpkdltlmisrtpvctcvvdvshedpevkfnwyvdgv
20 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietkiskakgqprepvytlppsrdeltknqvsltlvk
gfypsdiafewesngqpennyktppldsdgsfflyskltvdksrwwqgnvfscsvmhhealhnhytqkslsispkg

HuIgGMHncs1 (oligo for CSS)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac

25 HuIgGMHncs2 (oligo for SCS=ncs2)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg

HuIgGMHncs3 (oligo for SSC=ncs3)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g

30

hIgGWT3xba (3' oligo for above mutation introduction)

gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

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Vhser11: (oligo for Leu to Ser at VH11)

gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc

huIgG1-3' (3' oligo to amplify IgG1 C regions, 3' end of CH3)

5 gtc tct aga cta tca ttt acc cgg aga cag

huIgA/Gchim5 (oligo for pcr#1)

cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg

10 huIgAhg-5' (oligo for pcr#2)

gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc caa ct

huIgA3'

15 gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac

2H7 scFv IgAH IGG WT CH2CH3, 2H7 scFv with IgA hinge and WT CH2 and CH3

aagcttgcggccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggaca
20 aattgttctctccagctccagcaatcctgtctgtcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacat
gcactgggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtg
gcagtggggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggtttaacc
caccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtgctcggcggtgggtgatctggaggagtgaggagctctca
ggcttatctacagcagctctggggctgagctggtagggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacattaccagtt
25 acaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatacttctacaatcag
aagttcaagggaaggccacactgactgtagacaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
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cagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgcgcacctgaactcctggggggaccgtcagttt
tcctctccccccaaaaccaaggacacctcatgatctccggaccctgagggtcacatgcgtgggtgggtgacgtgagccacgaagacc
30 ctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaagccggggaggagcagtacaacagcacgta
ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaagccctccagc
ccccatcgagaaaacatctccaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgacc
aagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatgcgcgtggagtgaggagcaatgggcagccggaga

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mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsynhwyqqkpgsspkpwiapsn
lasgvparfsgsgsgtsysltisrveadaatyccqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytftsynmhwwkqtprrglewigaiypngdtsynqkfkkgatltvdksstaymqsltsedsavyfcarvvysns
5 ywyfdvwgtgtvtvssdqvpstptpsptpspcchprlslhrraledlllgseailtctltglrdasgvtftwtpssgksavqgp
pdrdlcgycysvssvlpgcaepwnhgtkftctaaypesktpltatlsksngtfrpevhlpppseealnelvtltclargfspkdvlrvwlq
gsgelprekyltwasrqpsqgtttfavtsilrvaedwkkgdftscmvgealplaftqktidrlagkpthvnsvsvmmaevdgtcy

0 tgatgacgcaggttcctcaactccacctaccocatctccctcaactccacctaccocatctccctcatgctgccacccc
cgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcc
tcagggtgcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgt
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ccgccaccctctcaaaatccggaaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctggccctgaacgagct
15 ggtgacgctgacgtgcctggcacgtggtctcagcccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgaga
agtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcactctgcggtggcagccgagga
ctggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggcccttcacacagaagaccatcgaccgcttggcgg
gtaaaccacccatgtcaatgtgtctgtgtcatggcggaggtggacggcacctgctactgataatctaga

Dqpvpstpptpspstpptpspcchprlslhrrpaedlllgscailtcttglrdasgvftwtpssgksavqgppdr
dlcgysvssvlpgcaepwnhgktftctaaypesktpitltsksngntfirpevhllpppseelalnelvtltclargfspkdvlvrwlqgsq
elprekyltwasrqepsqgtttfavtsilrvaaedwkkgdftscmvghlealplftqktidrlagkpthvnvsvvmaevdgtcy

agatctcaagaagatgaaaggattgttctgttgacaacaaatgtaagtgccccggattacttccaggatcatccgttc
ttccgaagatcctaattgaggacattgtggagagaaacatccgaattattgttctctgaacaacagggagaaalactctgatcccactcacc
attgagaaccagatttgtgtaccalltgtctgacctcagctgtaaaaaatgtgatcctacagaagtggagctggataatcagatagttactgcta
30 cccagagcaatatctgtgatgaagacagtgctacagagacctgtacacttatgacagaaacaagtgctacacagctgtgggtcccactcgtat
tatgtgtgtgtgagacaaaatgtgtggaacagccttaacccagatgcctgtatcctgactaatctaga

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rsqederivldnkckcaritsriirssedpnediverniriivplnnrenisdptsplrtrfvyhlsdlsckkdptev
eldnqivtatqsnicdedsatetcytdrnkcytavvplvyggetkmvetaltpdacyp

HUJCH5nl (J chain 5' primer)

5 gtt gtt aga tct caa gaa gat gaa agg att gtt ctt

HUJCH3 (J chain 3' primer-antisense)

gtt gtt tct aga tta gtc agg ata gca ggc atc tgg

10 4 carboxy terminal amino acids deleted from IgA CH3
GTCY

IgAH IgAT4 Human IgA tail, truncated (3T1)-(missing last 4 amino acids from
carboxy terminus)

15 tgatcagccagltccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccc
cgactgtcactgcaccgaccggccctcgaggacctgctcttaggtcagaagcgatcctcacgtgcacactgaccggcctgagagatgcc
tcaggtgtcaccttcacctggagccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctgtacagcgtgt
ccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgactgctgctaccccgagtccaagaccccgctaa
ccgccacccctctcaaatccggaacacattccggcccagggtccacctgctgccgccggcgtggaggagctggccctgaacgagct
20 ggtgacgctgacgtgcctggcacgtggcctcagcccaaggatgtgctggtcgtggctgcaggggtcacaggagctgccccgcgaga
agtacctgacttgggcatcccgaggagcccgagggcaccaccaccttgcgtgtgaccagcatactgcgcgtggcagccgagga
ctggaagaagggggacaccttctcctgcatggtgggcccagaggccctgccgctggccttcacagaagaccatcgaccgcttggcgg
gtaaacccaccatgtcaatgtgtctgtgtcatggcggagggtggactgataatctaga

25 IgAH IgAT4 Protein sequence:

Dqvpstppstpsptpspschprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdr
dlcgcyssvslpgcaepwnhgtfcttaaypesktpltatlsksngntfrpevhllpppseelalnelvtltclargfspkdvlvrlqgsq
elprekyltwasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghcalplafqtktidrlagkpthvvnsvvmaevd

30 HUIGA3T1 (Oligo 3': to delete 4 amino acids at carboxy end of IgA CH3)
gtt gtt tct aga tta tca gtc cac ctc cgc cat gac aac aga cac

HUIGA3T2: (oligo to delete 14 aa at end of IgA -T4)

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gtt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt

NT

2H7 scFv IgAH IgAT4

5 (2H7 scFv IgA 3T1 construct)--truncates the CH3 domain at the 3'end

aagcttgccgccatggatittcaagtgcagatttcagcttcctgctaatactgcttcagtcataattgccagaggaca
aattgttctctcccagctccagcaatcctgtctgcattctccaggggagaagggtcacaatgacttcagggccagctcaagtgtaagtacat
gcactgggtaccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtg
gcagtggtgtggtggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaacc
10 caccacgttcgtgctgggaccaagctggagctgaagatggcggtggctcggcggtgggtggtatcggaggaggtgggagctctca
ggcttatctacagcagctctggggtgagctggtagggcctggggtcagtggaagatgctctgcaaggcttctggctacacattaccagtt
acaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatacttctacaatcag
aagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
gtctattttgtgcaagagtgggtactatagtaactcttactgggtacttcgatgtctggggcacaggaccacggtcaccgtctcttctgatca
15 gccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcactgcaccgacc
ggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctgg
acgccctcaagtgggaagagcgtgttcaaggaccacctgacctctgtggctgctacagcgtgtccagtgctcctgccggggtgt
gccgagccatggaaccatgggaagaccttacttgcactgctgcctaccccgagtccaagaccccgctaaccgccacctctcaaaatcc
ggaaacacattccggcccgagggtccacctgctgccgccgctggaggagctggccctgaacgagctggtgacgtgacgtgcctgg
20 cacgtggctcagccccaaaggatgtgctggtctgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttggcatccc
ggcaggagcccagccaggggcaccaccacctcgtgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacacct
tctctgcatggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcggtgaaaccacccatgtcaatg
tgtctgttgcacggggaggtggactgataatctaga

25 AA

2H7 scFv IgAH-T4

mdfqvqifsfllisasviiargqivlsqpailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasn
lasgvparfsgsgtsysltisrveadaatyycqwsfnppfagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytfsynmhwwkqtpqrqglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltedsavyfcarvvyysns
30 ywyfdvwgtgtvtvssdqpvpsptpspsptpspschprlsrlhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp
pdrdlcgcysvssvlpgaepwnhgktftctaaypesktpitltsksgntfrpevhllpppseelalnelvtlclargfspkdvlvrvlq
gsqelprekyltwasrqepsqgtttfavlsvaaedwkkgtfscmvghealplafktidrlagkpthvnsvsvmaevd

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14 amino acids deleted from IgAH-T4 (so that total of 18 amino acids deleted from wild type IgA CH3)

PTHVNVSVVMAEVD

5

IgAH IgA-T18 (Human IgA Tail truncated, 3T2)

Tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgtgccaccc
ccgactgtcactgcaccgaccggccctcaggacctgtcttaggttcagaagcgcacgtgcacactgaccggcctgagagatgc
ctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgtgttcaaggaccacctgaccgtgacctctgtggctgtacagcgtg
10 tccagtgtcctgccgggtgtgccgagccatggaaccatgggaagaccttcactgtcactgtgcctaccccgagtccaagaccccgcta
accgccaccctctcaaaatccggaacacattccggcccagggtccacctgtgccgccgccgtcggaggagctggccctgaacgagct
ggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgaga
agtacctgacttgggcatccggcaggagcccagggcaccaccaccttcgctgtgaccagcactatgcgcgtggcagccgagga
ctggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgg
15 gtaaa

IgAH IgA-T18 Protein sequence:

dqvpstptpsptptpspschprlslrpaledlllgseailtctltglrdasgytftwtpssgksavqgppdrd
lccgysvssvlpgcaepwnhgktftctaaypesktpitailsksgntfrpevhllpppseelalnelvtltclargfspkdvivrwlqgsqe
20 lprekyltwasrqpsqgtttfavtsilrvaedwkkgdftscmvghaelplafktidrlagk

NT

2H7 scFv IgAH IgAT18: (Human IgA Tail truncated, 3T2.)

aagcttgccgcatggattttcaagtgacagatttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca
aattgttctctccagctctccagcaatctgtctgcatctccaggggagaagggtcacatgactgcagggccagctcaagtgaagtacat
gcactggtaccagcagaagccaggatcctccccaaccctggatttatgcccacccaacctggcttctggagtcctgctcgttcagtg
gcagtggtgtggacaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggatttaacc
caccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcgggcgggtggatctggaggaggtgggagctctca
30 ggcttatctacagcagctgagggtgagctgggtgaggcctggggcctcagtgaaagatgtcttgcaggccttctggctacacattaccagtt
acaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatacttctacaatcag
aagttaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg
gtctatttctgtgcaagagtgggtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccacggtcaccgtctctctgatca

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gccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcactgcaccgacc
ggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctgg
acgccctcaagtgggaagagcgtgtcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgt
gccgagccatggaacatgggaagaccttcacttgcactgtgctaccccgagtccaagaccccgtaaccgccacctctcaaaatcc
5 ggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgtgacgtgcctgg
cacgtggctcagcccaaggatgtgctggtcgtgctgcagggggtcacaggagctgccccgcgagaagtacctgacttgggcatccc
ggcaggagcccagccagggcaccaccaccttgcctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacacct
tctcctgcatggtgggccacgaggccctgccgtggccttcacacagaagacctcgaccgcttggcgggtaaa

0

AA:

2H7 scFv IgAH IgAT18:

mdfqvqifsfllisasviiargqivlsqpailsaspgekvmtcrasssvsymhwyqqkpgsspkpwiypsn
lasgvparfsgsgtsysltisrveadaatyycqwsfnpptfgagtkleldkgggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytfsynmhwwkqtrqglewigaiypngdtsynqkfkgtatltvdksstaymqlssltsedsavfcarvvyysns
15 ywyfdvwgtgtvtvssdqvpstpptpsptpspschprlslhrrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp
pdrldgcysvssvlpgaepwnhgktftctaaypesktp|tatlsksntfrpevhlpppseelalnelvtltclargfspkdvlvrwlq
gsqelprekyltwasrqepsqgtttfavtsilrvaedwkkgdtfscmvghealplafqtktidrlagk

20

CTLA-4 IgG WTH WTCH2CH3 (Human-oncoMLP-CTLA4EC-hIgGWT)

Nucleotide sequence:

gcaacctacatgatggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtg
aacctcactatccaaggactgagggccatggacacgggacttcatctgcaagtgaggctcatgtaccaccgccatactacctgggc
ataggcaacggaacccagatttatgtaattgatccagaacctgcccagattctgatcaacccaaatcttgtgacaaaactcacacatgccca
25 ccgtgcccagcacctgaactcctggggggaccgtcagtcttctcttcccccaaaacccaaggacacctcatgatctccgggaccttg
aggtcacatgcgtgggtgggacgtgagccacgaagacctgaggtcaagtccaactggtagctggacggcgtggaggtgcataatgcc
aagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggca
aggagtacaagtgaagggtccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
acaggtgtacacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatcccagcga
30 catcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccact
acacgcagaagagcctctccctgtctccgggtaaatga

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CTLA-4 IgG WTH WTCH2CH3 Protein sequence:

mgvlltqrllslvlallfpasmamhvaqpavvlassrgiasfvceyaspgkatevrvtlrqadsqvtevaa
tymmgneftlddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpsdqpkscdkthtppc
papellggpsvflfppkpkdtlmsirtpetvcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwl
5 gkeykckvsnkalpapietiskakgqprepvytlppsrdeitknqvslclvkgfypsdiavewesngqpennyktpvldsdgs
fflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgk

Human OncoM leader Peptide+CTLA4 EC (Bcll)

Atgggggtactgctcacacagaggacgtgctcagctgtgctccttgactcctgtttccaagcatggcgagcatggc
0 aatgcacgtggcccagcctgctgtggtactggccagcagccgagcatcgccagcttgtgtgtgagtatgcattccaggcaaagccact
gaggtccgggtgacagtgcctcggcaggctgacagccaggtgactgaagtctgtcggcaacctacatgatggggaatgattgacctc
ctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacgggactctaca
tctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatccagaaccgtgcc
agattctgatcaa

5

Human OncoM leader Peptide+CTLA4 EC Peptide sequence:

mgvlltqrllslvlallfpasmamhvaqpavvlassrgiasfvceyaspgkatevrvtlrqadsqvtevaa
tymmgneftlddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpsdq

20

Human OncoM leader peptide nucleotide

atgggggtactgctcacacagaggacgtgctcagctgtgctccttgactcctgtttccaagcatggcgagcatg

Human OncoM leader peptide sequence:

Mgvlltqrllslvlallfpm

25

NT

Human CTLA4 EC (no LP)

Gcaatgcacgtggcccagcctgctgtggtactggccagcagccgagcatcgccagcttgtgtgtgagtatgc
ctccaggcaaagccactgaggtccgggtgacagtgcctggcaggctgacagccaggtgactgaagtctgtcggcaacctacatgacg
30 gggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccat
ggacacgggactctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaacccagatttatgtaatt
gatccagaaccgtgccagattct

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AA

Human CTLA4 EC (no LP)

Amhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvtevcaatymtgneltfddsictgtssgnq
vnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpds

5

NT

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Atgggggtactgctcacacagaggacgtgctcagtcgtgctccttgccactcctgtttccaagcatggcgagcatggc
aatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatgccagctttgtgtgtgagtagtcatcaggc
10 gaggtccgggtgacagtgttcggcaggctgacagccagggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacctt
ctagatgattccatctgcacgggcacccctcagtggaatcaagtgaacctcactatccaaggactgagggccatggacacgggactctaca
tctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagattatgtaattgatccagaaccgtgcc
agattctgatcaacccaaatctctgacaaaactcacatccccaccgtccccagcacctgaactcctgggggagtcgtcagtcctctt
ccccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtgggtgacgtgagccacgaagaccctgag
15 gtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagccgaggaggagcagtacaacagcacgtaccgtg
tggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtcctcaacaagccctccagcccc
atcgagaaaacaatctcaaaagccaaaggcagccccgagaaccacaggtgtacaccctgccccatccgggatgagctgaccaaga
accaggtcagcctgacctgcctgggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaa
ctacaagaccacgcctcccgctggacitccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggg
20 gaacgtctctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

AA

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Mgvlltqrllslvlallfpsmasmamhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvtevca
25 atymmgneftfddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpdsdpkssdkthtsp
spapellggssvflfppkpkdtlmisrtpevtcvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwn
gkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvslclvkgfypsdiavewesngqpennykttpvldsdgs
fflyskltvdksrwqqgnvfscsvmhcalnhhytqkslspsgk

30

CTLA-4 IgAH IgACH2CH3 (Human-oncoMLP-CTLA4EC-IgA)

Nucleotide sequence:

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atgggggtactgtctcacacagaggacgtgctcagctgtgctccttgcactcctgtttccaagcatggcgagcatggc
aatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatgccagctttgtgtgtgagtatgcattccaggcaaagccact
gaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgaccttc
ctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctcactatccaaggactgagggccatggacacgggactctaca
5 tctgcaaggtggagctcatgtacccaccgccatactacctgggcataaggcaacggaacccagatttatgtaattgatccagaaccgtgcc
agattctgatcagccagttccctcaactccactacccatctccctcaactccactacccatctccctcatgtgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtc
accttcacctggacgccctcaagtgggaagagcgctgttaaggaccacctgaccgtgacctctgtggctgtacagcgtgtccagtgtcc
tgccgggctgtgccgagccatggaacctgggaagaccttactgtcactgctgcctacccgagccaagaccccgtaaccgccacc
0 ctctcaaaatccggaacacattccggcccagggtccacctgtgctgcggccgctcgaggagctggccctgaacgagctggtgacgct
gacgtgctggcacgtggtcctcagccccaaagatgtgctgttgcgtgctgcagggggtcacaggagctgccccgcgagaagtacctga
cttgggcatccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcactatgcgcgtggcagccgaggactggaagaa
gggggacaccttctcctgcatgtgtggccacgagggcctgcccgtggccttcacacagaagaccatgaccgcttggcggttaaccca
cccattgtcaatgtgtctgtgtcatggcggaggtggacggcacctgctactgataatctaga

5

CTLA-4 IgAH IgACH2CH3 Protein sequence:

mgvlltqrllslvlallfpsmasmamhvaqpavvlassrgiasfvcyaspkgatevrvtvlrqadsqvteveaa
tymmgneftlfdstictgssgnqvnlitqglramdglyickvelmypppylgigngtqiyvidpepcpsdqpvpstptpspst
pptpspcchprlslhrpaeldlllgseailctltglrdasgvflwtpssgksavqgppdrldgcysvssvlpgcaepwnhgtftcta
20 aypesktpltatlsksgntfrpevhllpppseelalnelvtltclargfspkdvlvrlwlgsgqlprekyltwasrqepsqgtttfavtsilrva
aedwkkgdtdfscmvghcalplftktidrlagkpthvvnsvvmaevdgtcy

CTLA-4 IgAH IgA-T4 (Human-oncoMLP-CTLA4EC-IgA3T1)

Nucleotide sequence:

25 atgggggtactgtctcacacagaggacgtgctcagctgtgctccttgcactcctgtttccaagcatggcgagcatggc
aatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatgccagctttgtgtgtgagtatgcattccaggcaaagccact
gaagggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgaccttc
ctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctcactatccaaggactgagggccatggacacgggactctaca
tctgcaaggtggagctcatgtacccaccgccatactacctgggcataaggcaacggaacccagatttatgtaattgatccagaaccgtgcc
30 agattctgatcagccagttccctcaactccactacccatctccctcaactccactacccatctccctcatgtgtgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtc
accttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgtacagcgtgtccaggtgtcc
tgccgggctgtgccgagccatggaacctgggaagaccttactgtcactgctgcctacccgagccaagaccccgtaaccgccacc

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ctctcaaaatccggaaacacattccggcccagggtccacctgctgccgccgctcggaggagctggccctgaacgagctggtgacgct
gacgtgcctggcacgtggcttcagccccaaaggatgtgctggctcgtggctgcagggggtcacaggagctgcccgcgagaagtacctga
cttgggcatcccgccaggagcccagccagggcaccaccaccttcgtgtgaccagcatactgcgcgtggcagccgaggactggaagaa
ggggggacacctctcctgcatggtggggccacgaggccctgccgtggccttcacacagaagaccatcgaccgcttggcgggtaaaccca
5 cccatgtcaatgtgtgtgtcatggcggagggtggactgataatctaga

CTLA-4 IgAH IgA-T4 Protein sequence:

Mgvlltqrllslvlallfpsmasmamhvaqpavvlassrgiasfvceyaspgkatevrvtlvrqadsqytevca
atymmgneltfddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpdsdqvpstptpsps
10 tpptpspscchprlslhrpaledlllgseailtctltlrdasgvtftwtpssgksavqppdrdlcgysvssvlpgaepwnhgtftcta
aypesktpitatisksngtfrpevhllpppseelalnelvtltclargfspkdvlvrlwlgsgqlprekyltwasrqepsqgtttfavtsilrva
aedwkkgdtfscmvghealplafqtidrlagkpthvnsvvmaevd

NT

15 human IgG1 CH2 with 238 mutation pro→ser

cctgaactcctggggggatcgtcagttcttcttcccccaaaacccaaggacacctcatgatctcccgaccct
gaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgaggagagcagtacaacagcacgtaccgtgtggtcagcgtcctcacgtcctgcaccaggactggctgaatggc
aaggagtacaagtgcaaggtctccaacaaagccctccagccccatcgagaaaaccatctccaaagccaaag

20

AA

human IgG1 CH2 with 238 mutation pro→ser

pellggssvflfpkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevlnaktkpreeqynstyrvvs
vltvlhqdwlngkeykckvsnkalpapiektiskak

25

Amino acids surrounding Pro to Ser in CH2

PAPELLGGPS

Amino acids surrounding Pro to Ser in CH2

30

PAPELLGGSS

HIgE5Bcl

gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc

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g t t g t t t c t a g a t t a a c t t t t a c c g g g a t t t a c a g a c a c c g c t c g c t g g

ggt gtt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

human IgE Fc (CH2-CH3-CH4) ORF:

25

human IgE Fc (CH2-CH3-CH4) ORF:

148

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IFhIgGwtBcl5

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc

(63 mer)

5

hIgGWT3xba

gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant

10 ***SSS'***

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa

ctc ctg ggt gga ccg tca gtc ttc c

NT

15

1D8 VH

cagggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctg

gggtctcattaaccagcgatgggtgacactggattcgacagcctccaggaaagggtctggaatggatgggaataatattatgatggaggc

acagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaatcaacagctgcaaaact

gatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctct

20

AA

1D8 VH (no leader)

qvqlkeagpglvqptqtlsltctvsgfsltsdgvhwirppgkglewmgiyydggtdynsaiksrlsrsrdsks

qvflkinslqtdtdamyycarihfdywgqgvmvtvss

25

NT

1D8 VL (no leader)

gacattgtgctcactcagctccaacaaccatagctgcatctccaggggagaaggtcacatcacctgccgtgccag

ctccagtgtaagttacatgtactgggtaccagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctgcttctggagtt

30

cca aatcgcttcagtgagtggtctgggacctcttattctctgcaatcaacaccatggagactgaagatgctgccacttattactgtcagc

agtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

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AA

1D8 VL

divltqspttiaaspgekvtitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsgsgsgtsyslaint
metedaatyycqqwsstpltfsgsgtleikr

5

NT

1D8 scFv

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
10 gtgctcactcagtcaccaacaaccatagctgcatctccaggggagaaggtcacatcacctgccgtgccagctccagtgtaagttacatgta
ctggtagcagcagaagtcaggcgctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtgga
gtgggtctgggacctcttattctctgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgct
cacgttcgggtctgggaccaagctggagatcaaacgggtggcgggtggctcggcggtgggtcgggtggcgccgatctcaggtg
cagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaaccagcgatg
15 gtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagattataattcagcaatta
aatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaatcaacagctctgcaaactgatgacacagccatgtattac
tgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtcctctgatca

AA

20

1D8 scFv

mdfqvqifsfllisasvimsrgvdivltqspttiaaspgekvtitcrasssvsymywyqqksgasplkiwydtskl
asgvpnrfsgsgsgtsyslaintmetedaatyycqqwsstpltfsgsgtleikrgggsgggsgggsgqvqlkeagpglvqptqlsl
ctvsgfsltsdgvhwirppgkglewmgiyydgtdynsaiksrlsirdtsksqvflkinslqtdtdamyycaihfdywgqgv
vtvss

25

NT

1D8 scFv IgG WTH WTCH2CH3

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
30 gtgctcactcagtcaccaacaaccatagctgcatctccaggggagaaggtcacatcacctgccgtgccagctccagtgtaagttacatgta
ctggtagcagcagaagtcaggcgctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtgga
gtgggtctgggacctcttattctctgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgct
cacgttcgggtctgggaccaagctggagatcaaacgggtggcgggtggctcggcggtgggtcgggtggcgccgatctcaggtg

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cagctgaa ggaggcaggacctggcctgggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttcattaaccagcgatg
gtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatattatgatggaggcacagattataattcagcaatta
aatccaga.ctgagcatcagcaggacacctccaagagccaagtttttaaaaaatcaacagtctgcaaactgatgacacagccatgtattac
tgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatcaggagcccaaatcttgacaaaactcacaca
5 tggccaccgtggccagcacctgaactcctggggggaccgtcagtcttctcttcccccaaaacccaaggacacctcatgatctcccga
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atggcaaggagtacaagtgaaggtctccaacaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccg
agaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctggtcaaaggcttctatcc
10 cagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctc
cttctctcttacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcac
aaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15

AA

1D8 scFv IgG WTH WTCH2CH3

mdfvqifsfllisasvimsrgvdivltqspptiaaspgekvtitcrasssvsymywyqqksgaspkfwiydtskl
asgvpnrfsgsgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsgggsgggsgsqvqlkeagpglvqptqlsl
ctvsgfsltsdgvhwirpppgkglewmgiiyydggtdynsaiksrlsirdtsksqvflkinslqtdtdamyyarihfdywgqgvm
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qynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakqprepvytlppsrdeftknqvsltelvkgfypsdiavewe
sngqpenryktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

25

NT

1D8 scFv IgG MTH MTCH2CH3-CD80

aagcttatggatttcaagtgcagattttcagcttctcgtaatcagtgcttcagtcataatgtccagaggagtcgacatt
gtgtcactcagtcaccaacaaccatagctgcacatccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgta
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gtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatattatgatggaggcacagattataattcagcaatta
aatccaga.ctgagcatcagcaggacacctccaagagccaagtttttaaaaaatcaacagtctgcaaactgatgacacagccatgtattac

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10 aatggaattttgtgatagctgctgacctaactgcttgcctcccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgt
acgcctgtataaatcgata

AA

1D8 scFv IgG MTH MTCH2CH3-CD80

15 mdfqvqifsfllisasvimsrgvdivltqspstiaaspgekvtitrasssvsymywyqqksgasplkiwydtskl
asgvpnrfsgsgsytyslaintmetedaatyycqqwsstpltfsgtkleikrgggsgggsgggsgvqlkeagplvqptqtlst
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NT

1D8 scFv IgG WTH WTCH2CH3-CD80

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AA

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NT

Anti human CD3 scFv WTH WTCH2CH3

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AA

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NT

20 *2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3*
2h7-40.2.220Ig + restriction sites
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15

AA

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3

2H7-40.2.220Ig

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NT

30 5B9 VH (includes the VH leader peptide)

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5B9 VH missing the leader:

5 cagggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtctctg
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gtctcctca

10

AA

5B9 VH (includes leader peptide)

MAVLGLLFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLITCTVSGFSLTTY
AVHWVRQSPGKGLEWLGVIWSSGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDT
15 AIYYCARNGGDNYPPYYAMDYWGQGTSTVTVSS

5B9 VH no leader peptide

QVQLKQSGPGLVQSSQSLITCTVSGFSLTTYAVHWVRQSPGKGLEWLG
VIWSSGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPPYYA
20 MDYWGQGTSTVTVSS

NT

5B9 VL

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30

AA

5B9 VL

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AA

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30 NT

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80)

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20 AA
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30 NT
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AA

2H7 scFv MH (SSS) MCH2WTCH3

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20 l a s g v p a r f s g s g s t s y s l t i s r v e a d a t y y c q w s f n p p t f g a g t k l e l k d g g s g g g s g g g s s q a y l q q s g a e l v r p g a s v k
m s c k a s g y t f s y n m h w v k q t p r q l e w i g a i y p n g n d t s y n q k f k g a t l t v d k s s t a y m q l s s l t s e d s a v y f c a r v v y s n s
y w y f d v w g t g t t v t v s s d e p k s s d k t h t s p p p a p e l l g g s s v f l f p p k p k d t l m i s r t p e v t c v v d v s h e d p e v k f n w y d g v
e v h n a k t k p r e e q n s t y r v v s v l t v l h q d w l n g k e y k c k v s n k a l p a p i e k t i s k a k g p r e p q v y t l p p s d e l t k n q v s l t c l v k
g f y p s d i a v e w e s n g q p e n n y k t t p p v l d s d g s f f l y s k l t v d k s r w q q n v f s c s v m h e a l h n h y t q k s l s l s p g k
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NT

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g a t g a c g c a g g c t g c a t t c c a a t c c a g t c a c t c t t g a a c a t c a g c t t c a t c t c t g c a g g t c t a g t a a g a g t c t c t a c a t a g t a a t g g c
30 a t c a c t t a t t i g t a t t g t a t c t g c a g a a g c c a g g c c a g t c t c t c a g t c c t g a t t a t c a g a t g c c a a c c t t g c c t c a g g a g t c c c a g a c a g
g t t c a g t a g c a g t g g g t c a g g a a c t a t t t c a c a c t g a g a a t c a g c a g a g t g g a g g c t g a g g a t g t g g g t g t t a t t a c t g t g c t c a a a t c t
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AA

15

5B9 scFv MTHWTCH2CH3

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25

Human IgG1 hinge mutations

30

2H7 scFv- MTH (CSS) WTCH2CH3

Nucleotide:

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2H7 scFv- MTH (CSS) WTCH2CH3 protein:

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25 2H7 scFv- MTH (SCS) WTCH2CH3:

Nucleotide:

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2H7 scFv- MTH (SCS) WTCH2CH3 Protein:

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2H7 scFv- MTH (SSC) WTCH2CH3:

Nucleotide:

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2H7 scFv- MTH (SSC) WTCH2CH3 Protein:

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ggt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

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HIgGMHcys2

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HIgGMHcys3

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NT

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2H7 scFv MTH (CCS) WTCH2CH3

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20

AA

2H7 scFv MTH (CCS) WTCH2CH3

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30

NT

HuIgAHIgA-T4-ORF

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PCT/US2003/024918

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AA

10 HuIgAHIgA-T4-ORF

Dqpvpstptpsptptpspschprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdr
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NT

15 HuIgAHIgA-T4-ORF

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25

AA

HuIgAHIgA-T4-ORF

dqpvpstptpsptptpspschprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrd
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30 lprekyltwasrqepsqgtttfavtsilrvaaedwkkgtfscmvghcalplafktidrlagkpthvnsvsvmaevdadpsn

NT

1D8-IgAH IgA-T4-CD80

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PCT/US2003/024918

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20

AA

1D8 scFv IgAH IgA-T4-CD80

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25 a s g v p n r f s g s g t s y s l a i n t m e t e d a a t y y c q q w s s t p l t f g s g t k l e i k r g g g s g g g s g g g s q v q l k e a g p g l v q p t q t l s t
c t v s g f s l t s d g v h w i r q p p g k g l e w m g i i y d g g t d y n s a i k s r l s i s r d t s k s q v f l k i n s l q t d d t a m y c a r i h f d y w g g v m
v t v s s d q p v p s t p t p s p s t p t p s p s c h p r l s h r p a l e d l l l g s e a i l t c t l t g l r d a s g v t f t w p s s g k s a v q g p p d r d l c g c y s v s s
v l p g c a e p w n h g k t f t c t a a y p e s k t p l t a t l s k s g n t f r p e v h l p p p s e l a l n e l v t l t c l a r g f s p k d v l v r w l q g s q e l p r e k y l t w
a s r q e p s q g t t t f a v t s i l r v a e d w k k g d t f s c m v g h e a l p l a f t q k t i d r l a g k p t h v n v s v m a e v d a d p s n n l p s w a i t l i s v n
30 g i f v i c l t y c f a p r c r r r n e r l r r e s v r p v

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human IgE Fc (CH2-CH3-CH4) ORF:

tgatcacgtctgtccagggaacttcacccgccaccgtgaagatcttacagtcgtcctgcgacggcgggggcacc
tccccccgaccatccagtcctgtgcctcgtctctgggtacacccagggaactcaacatcacctggctggaggacgggcaggtcatgg
acgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctg
5 tcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccagaagtggtgcagattccaacccgagaggg
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15

AA

human IgE Fc (CH2-CH3-CH4) ORF:

dhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmdvdlstastqegelastqseltl
sqkhwlslrdtytcqvttyqghtfedstkkcadnsnprgvsaylsrpspfdlfrksptitclvdlapskgvnltsrasgkpvnhstrikee
20 kqrngltltvstlpvgtrdwiegetyqcrvthphlpralmrstktsgpraapevyafatpewpgsrdrktlacliqnfmnpedisvqwlh
nevqlpdrhsttqprktksgffvfrlevtraeweqldeficravheaaspsqtvqravsvnpgkadps

25 NT

1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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gtgggtctgggaccttattctctgcaatcaacacctggagactgaagatgctgccattattactgtcagcagtgaggtagtactccgct
30 cacgttcgggtctgggaccaagctggagatcaaacgggggtggcggtggctcggcggtggtgggtggcggggatctcaggtg
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PCT/US2003/024918

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agaaggggaaagtgtacgcct g tataaatcgata

15

AA 1D8-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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ctvsgfsltsdgvhwirppgk glewmgiyydgtdynsaiksrlsisrdtsksqvfkinslqtdtdamyycarihfdywgqgvm
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rtytcqvyqghtfedstkkca d snprgvsaylsrspfdl firkspitclvvdlapskgtnltwsrasgkpvnhstrkeekqrngltvt
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errnerlrresvrpv

25

NT

5B9-IgA H IgA-T4-CD80

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30 gatgacgcaggtgcatttccaatccagtcactcttgaacatcagcttccatctcctcaggtctagtaagagtctcctacatagtaatggc
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15 ctgtataaatcgatac

AA

5B9-IgAH IgA-T4-CD80

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NT

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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10 cgtgaacctgacctggtcccggccagtggaagcctgtgaacctccaccagaaaggaggagaagcagcgcaatggcacgttaacc
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20

AA

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

mrfsaqlllglvlwipgstadivmtqaafsnptlgtasiscrssksllhsngitylywylqkpgqspqlliyqms
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25 sitctvsgfsltyavhwvrqspgkglewlgviwsggitynaafisrlsitkddsksqvffkmnslqpnndtaiyyarnngdnpyyy
amdywgqgtsvtvssdhvc srdftpptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqymdvdlstasttqegelastqs
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keekqrmgtltvtstlpvgrd wiegetyqervthphlpralmrstktsgpraapevyafatpewpgsrdkrtlaclqnfmpedisvq
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30 vicltycfaprerrrrnerlrresvrpv

NT

2e12-scFv-IgAH IgA-T4-CD80

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aagcttaaggatttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
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20 ccctgtataaatcgatac

AA

2e12-scFv-IgAH IgA-T4-CD80

25 mdfqvqifsfllisasvimsrgvdivltqspaslavslgqratisrasesveyyvtslmqwyqqkpgqppkllis
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psqslsitctvsgfsltgygvnwvrrppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvlkmnslqtdtdaryycardgys
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30 rwlqgsqelprekyltwasrcqpsqgtttfavtsilrvaedwkkgdftscmvghalplafktidrlaglkpthvnsvvmaevda
dpsnnllpswaitlisvngifviccltycfaprerrrrnerlrresvrpv

NT

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2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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25

AA

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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aasnvesgvparfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkespglva
30 psqslsitctvsgfsltygvnwvrrppgkglewlgmiwgdstdynsalksrslsitkdnsksqvfllkmnslqtdtdaryycardgys
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nhstrkeekqrngtltvtstlpvgtrdwegetyqcrvthphlpralmrsttktsgpaaapevyafatpewpgsrdkrtlaclqnfmpe

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isvqwlhnevqlpdarhsttqprktkgsgrffvfrlevtraeweqkdeficravheaaspsqtvqravsvnpkgadpsklpswaitlisv
ngifviccltycfaprerrmerlrresvrpv

NT

5 500A2 scFv

atgtgtatacatctcagctcctgggctttactcttctggatttcagcctccagaagtgacatagtgctgactcagactc
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15

AA

500A2 scFv

mlytsqllglllfwisarsdivltqtpatlslipgervtmtcktsqniqtilhwyhqpkeapralikyasqsipgip
srfsgsgsetdftlsinnlepddigiyyccqsrswpvtfpgptkleikrgggsgggsgggsgqvkllqsgselgkpgasvklckts
20 gyiftdhyiswvkqkpgeslqwi gnvygnggtsynqkfqgkatltvdkisstaymelsltsedsaiyyccarrpvatghamdywg
qgiqvtvssd

5' oligo:

25

Name : hIgAbcl5

Sequence : GTTGTTGATCAGCCAGTTCCTCAACTCCACCTACC

3' oligo:

30

Name : IgA3BB

GTTGTTTTTCGAAGGATCCGCGTCCACCTCCGCCATGACAACAGA

5' oligo:

Name : IgGWT3

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GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

3' oligo:

Name : hIgGWT5

5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACACACATG

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5' oligo:

Name : FADD5

Sequence :

5 GTTGTGGATCCTTCGAACCCGTTCTGGTGCTGCTGCACTCGGTGTCG

3' oligo:

Name : FADD3

Sequence :

10 GTTGTTCATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

FADD-CSSCFV:

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15 acgacctggagcccgggcacaccgagctcctgcgcgagctgctgcctccctgcggcgccacgacctgctgcggcgctgcagcactt
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20 agacgcacatctacctccgaagcgtcctgataactcgagatcgataacaac

Peptide sequence:

vdpsnpflvllhsvssslsssttelkflclgrvgrklervqsgldlfsmllleqndlepghtellrellaslrhdllrrv
ddfeagaaagaapgeedlcaafnvi cdnvgkdwrllarqlkvsdtkidsiedryprnltervreslriwknktenatvahlvgalrscq
25 mnlvadlvqevqqardlqnrsgamspmswnsdastseas

Name : HCD28tm5B

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTGGTGGTGGCTT
30 GCTATAGCTTG

Name : HCD28tm3S

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GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCT
ATAGCAAGCCAG

5 HCD28tm5'
GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT
HCD28tm3'
GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

10 HCD80tm5'
GTTGTGGATCCTCCTGCTCCCATCCTGG
HCD80tm3'
GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

15 Name : MFADD5BB
Sequence :
GTTGTGGATCCTTCGAACCCATTCCTGGTGCTGCTGCACTCGCTG

20 Name : MFADD3XC
Sequence :
GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

25 Murine FADD Nucleotide sequence (full length, but without flanking -Ig or
transmembrane sequences):

Gtggatcctcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgat
ggagctcaagttctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtgccctggacctgtcacggtgctgctgg
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30 agagactggaaaagactgcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagtctgagtg
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Murine FADD

vdpsnmdpflvllhslsgslsgndlmelkflcrervskrklerqvsgldlftvlleqndlerghtglrellaslrhdll
qrlddfeagtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprlservreslkvwknaekknasvaglvk
5 alrtcrlnlvadlveeaqesvsksenmspvlrdstvsstetp

Name : MCASP3-5
10 Sequence :
GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA
Name : MCASP3-3
Sequence :
GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT
15

Name : mcasp8-5
Sequence :
20 GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG
Name : mcasp8-3
Sequence :
GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG
25

Name : hcasp3-5
Sequence :
GTTGTGGATCCTTCGAACATGGAGAACAACACTGAAAACCTCAGTGGAT
Name : hcasp3-3
30 Sequence :
GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

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Name : hcas8-5

Sequence :

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

Name : hcas8-3

5 Sequence :

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

HuIgGMHWC

10 gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa
ctc ctg ggt gga ccg tca gtc ttc c

NT

15 2H7-human IgE (CH2-CH3-CH4)

aagcttgccgcatggattttcaagtgagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca
aattgttctctccagtcctcagcaatcctgtctgcatctccaggggagaaggtcacatgacttcagggccagctcaagttaagttacat
gcactggtagcagagaagccaggtatctccccaacccctggatttatgcccacccaacccctggcttctggagtcctgctcgttcagtg
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20 caccacgttcggtgctgggaccaagctggagctgaaaggtggcgggtgctcggggtggtggatctggaggaggtgggagctctca
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cacagcacgacgcagccccgaagaccaagggtccggcttcttcttctcagccgcctggaggtgaccagggccgaatgggagcaga
aagatgagttcatctgccgtgcagtcctgaggcagcgagccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaatgata
atctaga

5

AA

2H7 scFv IgE (CH2-CH3-CH4)

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiyapns
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10 ywyfdvwgtgtvtvsdhvcsrdftpptvkilqsscdggghfpptiqlclvsytpgtinitwledgqvmdivlstaattqegelastqs
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15

NT

2H7 scFv MH (SSS) MCH2WTCH3

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20 aattgttctctcccagtcctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgaattacat
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agcctgacctgcctggtaaggcttctatccagcgacatgcctggagtgaggagagcaatgggcagccggagaaactacaaga

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ccacgcctcccgctgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccgctgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

5

2H7 scFv MH (SSS) MCH2WTH3

mdfqvqifsflisaviiargqivlsqpailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiapsn
lasgvparfsgsgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgyftsynmhwwkqtrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysns
ywyfdvwtgtttvssdqepkssdkthtspspapellggssvflfppkpkdtlmisrtpetvctvvdvshedpevkfnwyvdgv
10 evhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltclvk
gfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslspsgk

NT

5B9 scFv MTHWTH2CH3

15

aagcttgccgccatgaggttctctgctcagcttctggggctgctgtgctctggatccctggatccactgcagatattgt
gatgacgcaggctgcatttccaatccagtcactcttggaaacatcagcttccatctcctgcaggctctagtaagagtctcctacatagtaattggc
atcacttatttgattggatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtccagacag
gttcagtagcagtggtgaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttactgtgctcaaatct
agaactccgctcacgttcggtgctgggaccaagctggagctgaacgggggtggcgggtggctcggggcgggtgggtgggtggcggc
20 ggatcgtcacagggtgcagctgaagcagtcaggacctggcctagtgagtcctcacagagcctgtccatcacctgcacagtctctggttctc
attaactacctatgctgtacactgggtcggcagctcctcaggaaagggtctggagtggctgggagtatatggagtgggtgaatcacagact
ataatgcagcttccatattcagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagcttgcacctaataacac
agccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtaaggaaacctcagtcaccgtctcc
tctgatcaggagcccaaatctctgacaaaactcacatccccaccgtcccagcactgaactcctggggggaccgtcagcttctctt
25 ccccccaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtggtgggtgagcgtgagccacgaagacctgag
gtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg
tggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccc
atcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctgaccaaga
accaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaa
30 ctaacagaccacgcctccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggg
gaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

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5B9 scFv MTHWTCH2CH3

mrfsaqllgllvlwipgstadivmtqaafsnptlgtasiscrskslhsngitylywylqkpgqspqlliyqms
nlasgvpdrfsssgsgtdflrisrveadvgvyycanlelpltfagtklelkrggggsgggsgggssqvqlkqsgpglvqssqsl
sitctvsgfslttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffkmnslqpndtaiyycarnggdnyppyy
5 amdywgqgtsvtvssdqepkssdkthtsppspapellggpsvflfppkpkdtlmisrtpetcvvvdvshedpevkfnwyvdgve
vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltelvkgf
ypsdiafewesngqpennyktppldsdgsfflyskltvdksrwqqgnvfscsvmhcalnhhytqkslsispk

10

From the foregoing, it will be appreciated that, although specific embodiments of
the invention have been described herein for the purpose of illustration, various modifications
may be made without deviating from the spirit and scope of the invention. Accordingly, the
15 present invention is not limited except as by the appended claims.

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CLAIMS

1. A binding domain-immunoglobulin fusion protein, comprising:
 - 5 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human
10 IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, and
15 (iv) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues;
 - (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to
20 the hinge region polypeptide; and
 - (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,
wherein:
 - (1) the binding domain-immunoglobulin fusion protein is capable of at least
25 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and
 - (2) the binding domain polypeptide is capable of specifically binding to an antigen.
- 30 2. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide, said immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine reside

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is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not mutated and one or both of said second and third cysteine residues is substituted or deleted; and c) an N-terminally truncated immunoglobulin heavy chain constant region polypeptide fused to said immunoglobulin hinge
5 region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

3. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding
10 domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from the group consisting of an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

4. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises
15 an immunoglobulin heavy chain variable region polypeptide, wherein said heavy chain variable region polypeptide is a human immunoglobulin heavy chain variable region polypeptide comprising a mutation at an amino acid at a location corresponding to amino acid position 11 in the first framework region of the heavy chain variable region.

20 5. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises a polypeptide having a sequence selected from the group consisting of SEQ ID NO:___ and SEQ ID NO:___.

6. The binding domain -immunoglobulin fusion protein of claim 3 wherein the
25 immunoglobulin variable region polypeptide is derived from a human immunoglobulin.

7. The binding domain -immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence.

30

8. The binding domain-immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide is derived from a murine immunoglobulin.

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9. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises:
 - (a) at least one immunoglobulin light chain variable region polypeptide;
 - (b) at least one immunoglobulin heavy chain variable region polypeptide; and
 - 5 (c) at least one linker polypeptide that is fused to the polypeptide of (a) and to the polypeptide of (b).
10. The binding domain-immunoglobulin fusion protein of claim 9 wherein the immunoglobulin light chain variable region and heavy chain variable region polypeptides are
10 derived from human immunoglobulins.
11. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least one polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___].
15
12. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___].
- 20 13. A binding domain-immunoglobulin fusion protein according to claim 9 wherein the linker comprises a glycosylation site.
14. The fusion protein of claim 13 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-
25 mannosylation site, a glypiation site and a phosphoglycation site.
15. The binding domain-immunoglobulin fusion protein of claim 1 wherein at least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide is derived from a human immunoglobulin heavy
30 chain.

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16. The binding domain-immunoglobulin fusion protein of claim 1 wherein the immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of an isotype selected from the group consisting of human IgG and human IgA.
- 5 17. The binding domain-immunoglobulin fusion protein of claim 1, 2 or 73 wherein the antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, L6, CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.
- 10 18. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain.
- 15 19. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain and at least one immunoglobulin variable region polypeptide.
- 20 20. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CTLA-4 extracellular domain.
- 25 21. The binding domain-immunoglobulin fusion protein of claim 2 or 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide.
- 30 22. The binding domain-immunoglobulin fusion protein of claim 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide.
23. A binding domain-immunoglobulin fusion protein, comprising:
 - (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

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(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,

5 wherein:

(1) the binding domain polypeptide comprises a CTLA-4 extracellular domain that is capable of specifically binding to at least one CTLA-4 ligand selected from the group consisting of CD80 and CD86,

(2) the immunoglobulin hinge region polypeptide comprises a polypeptide
10 that is selected from the group consisting of a human IgA hinge region polypeptide and a human IgG1 hinge region polypeptide,

(3) the immunoglobulin heavy chain CH2 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH2 constant region polypeptide and a human IgG1 heavy chain CH2 constant region
15 polypeptide,

(4) the immunoglobulin heavy chain CH3 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH3 constant region polypeptide and a human IgG1 heavy chain CH3 constant region polypeptide, and

(5) the binding domain-immunoglobulin fusion protein is capable of at least
20 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

24. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide comprises a human IgE hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human
30 IgE CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a human IgE CH3 constant region polypeptide

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wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and
- 5 (2) the binding domain polypeptide is capable of specifically binding to an antigen.

25. A binding domain-immunoglobulin fusion protein according to claim 24 that comprises a human IgE CH4 constant region polypeptide.

10

26. The binding domain-immunoglobulin fusion protein of claim 24 wherein the antigen is a tumor antigen.

27. A binding domain-immunoglobulin fusion protein, comprising:

15 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region polypeptide;

20

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;

25

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and

30

(d) a plasma membrane anchor domain polypeptide.

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28. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.
29. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.
30. The binding domain-immunoglobulin fusion protein of claim 29 wherein the cytoplasmic tail polypeptide comprises an apoptosis signaling polypeptide sequence.
31. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence is derived from a receptor death domain polypeptide.
32. The binding domain-immunoglobulin fusion protein of claim 31 wherein the death domain polypeptide comprises a polypeptide selected from the group consisting of an ITIM domain, an ITAM domain, FADD, TRADD, RAIDD, CD95 (FAS/ Apo-1), TNFR1 and DR5.
33. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence comprises a polypeptide sequence derived from a caspase polypeptide that is selected from the group consisting of caspase-3 and caspase-8.
34. The binding domain-immunoglobulin fusion protein of claim 27 wherein the plasma membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.
35. The binding domain-immunoglobulin fusion protein of claim 27 wherein the antigen that is present on an immune effector cell is selected from the group consisting of CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.
36. The binding domain-immunoglobulin fusion protein of claim 27 wherein the human IgG is human IgG1.

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37. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and

(d) a plasma membrane anchor domain polypeptide.

38. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.

39. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.

40. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.

30

41. The binding domain-immunoglobulin fusion protein of claim 37 wherein the human IgG is human IgG1.

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42. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide comprises a wild-type human IgA hinge region polypeptide;

5 (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human IgA CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of (i) a wild-type human IgA CH3 constant region polypeptide and (ii) a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain,

wherein:

(1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and

(2) the binding domain polypeptide is capable of specifically binding to an antigen.

20 43. The binding domain-immunoglobulin fusion protein of claim 42 wherein the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is selected from the group consisting of (i) a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:___ and (ii) a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:___.

25

44. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a llama CH2 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH2 constant region polypeptide, a llama IgG2 CH2 constant region polypeptide and a llama IgG3 CH2 constant region polypeptide; and

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(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a llama CH3 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH3 constant region polypeptide, a llama IgG2 CH3 constant region polypeptide and a llama IgG3 CH3 constant region polypeptide

wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction fixation of complement, and
- (2) the binding domain polypeptide is capable of specifically binding to an antigen.

45. A binding domain-immunoglobulin fusion protein according to claim 44 wherein the immunoglobulin hinge region polypeptide, the llama CH2 constant region polypeptide and the llama CH3 constant region polypeptide comprise sequences derived from a llama IgG1 polypeptide and wherein the fusion protein does not include a llama IgG1 CH1 domain.

46. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the hinge region polypeptide is mutated to contain a glycosylation site.

47. The fusion protein of claim 46 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site and a phosphoglycation site.

48. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of said binding domain polypeptide sequences is capable of specifically binding to an antigen.

49. A binding domain-immunoglobulin fusion protein, comprising:

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(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,

wherein:

(1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and

(2) the binding domain polypeptide is capable of specifically binding to an antigen.

50. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and

(d) a plasma membrane anchor domain polypeptide.

51. A binding domain-immunoglobulin fusion protein according to either claim 49 or claim 50 wherein the alternative hinge region polypeptide sequence comprises a polypeptide sequence

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of at least ten continuous amino acids that are present in a sequence selected from the group consisting of SEQ ID NOS: __ - __.

52. An isolated polynucleotide encoding a binding domain-immunoglobulin fusion protein
5 according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50.

53. A recombinant expression construct comprising a polynucleotide according to claim 52 that is operably linked to a promoter.

10 54. A host cell transformed or transfected with a recombinant expression construct according to claim 53.

55. A method of producing a binding domain-immunoglobulin fusion protein, comprising the steps of:

- 15 (a) culturing a host cell according to claim 54 under conditions that permit expression of the binding domain-immunoglobulin fusion protein; and
- (b) isolating the binding domain-immunoglobulin fusion protein from the host cell culture.

56. A pharmaceutical composition comprising a binding domain-immunoglobulin fusion
20 protein according to any one of claims 1, 2, 23, 24, 42, 44, 49 or 50 in combination with a physiologically acceptable carrier.

57. A pharmaceutical composition comprising an isolated polynucleotide encoding a binding
25 domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50 in combination with a physiologically acceptable carrier.

58. The use of a compound according to claim 1 in the preparation of a medicament for the treatment of a subject having or suspected of having a malignant condition or a B-cell disorder, comprising administering to a patient a therapeutically effective amount of a pharmaceutical
30 composition selected from the group consisting of the pharmaceutical composition of claim 56 and the pharmaceutical composition of claim 57.

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59. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of a B-cell lymphoma and a disease characterized by autoantibody production.

5 60. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis and an autoimmune disease.

61. The use of claim 58 wherein the malignant condition is selected from the group
10 consisting of melanoma, carcinoma and sarcoma.

62. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine residue is substituted or deleted and said third cysteine residue is not substituted or deleted.

15

63. A binding domain-immunoglobulin fusion protein according to claim 62 wherein said second cysteine residue is substituted and not deleted.

64. A binding domain-immunoglobulin fusion protein according to claim 63 wherein said
20 second cysteine is replaced with serine.

65. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said third cysteine residue is substituted or deleted and said second cysteine residue is not substituted or deleted.

25

66. A binding domain-immunoglobulin fusion protein according to claim 65 wherein said third cysteine residue is substituted and not deleted.

67. A binding domain-immunoglobulin fusion protein according to claim 66 wherein said
30 third cysteine is replaced with serine.

68. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine and said third cysteine residues are both substituted or deleted.

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69. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are substituted.

5 70. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are replaced with serine.

71. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy
10 chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

72. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy
15 chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

73. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen, said binding domain
20 polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide, wherein the leucine at position 11 in the first framework region of said heavy chain variable region polypeptide is substituted or deleted; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide; and c) an N-terminally truncated immunoglobulin heavy chain constant region
25 polypeptide fused to said immunoglobulin hinge region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

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FIG. 1A

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2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide→
 ~~~~~  
 M D F Q V Q I F S F L L I S A S  
 1 AAGCTTGCCG CC ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V<sub>L</sub>→  
 V I I A R G Q I V L S Q S P A I L S A S  
 61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W  
 121 CCAGGGGAGA AGGTCACAAT GACTTGCCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
 ~~~~~  
 Y Q Q K P G S S P K P W I Y A P S N L A
 181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I
 241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

S R V E A E D A A T Y Y C Q Q W S F N P
 301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker
 P T F G A G T K L E L K G G G G S G G G
 361 CCCACGTTCTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H→
 G S G G G G S S Q A Y L Q Q S G A E L V
 421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y
 481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y
 541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTATT

P G N G D T S Y N Q K F K G K A T L T V
 601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A
 661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W
 721 GTCTATTCTT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

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FIG.1B

BclI

-----human IgG1 Fc domain →

```
781  G T G T T V T V S D Q E P K S C D K T H
    GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

    T C P P C P A P E L L G G P S V F L F P
841  ACATGCCCAC CGTGCCACAGC ACCTGAACTC CTGGGGGGGAC CGTCAGTCTT CCTCTTCCCC

    P K P K D T L M I S R T P E V T C V V V
901  CCAAAACCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

    D V S H E D P E V K F N W Y V D G V E V
961  GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

    H N A K T K P R E E Q Y N S T Y R V V S
1021 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

    V L T V L H Q D W L N G K E Y K C K V S
1081 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

    N K A L P A P I E K T I S K A K G Q P R
1141 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

    E P Q V Y T L P P S R D E L T K N Q V S
1201 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

    L T C L V K G F Y P S D I A V E W E S N
1261 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

    G Q P E N N Y K T T P P V L D S D G S F
1321 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCCTGC TGGACTCCGA CGGCTCCTTC

    F L Y S K L T V D K S R W Q Q G N V F S
1381 TTCCTCTACA GCAAGTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

    C S V M H E A L H N H Y T Q K S L S L S
1441 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCTGTCT
```

XbaI

~~~~~

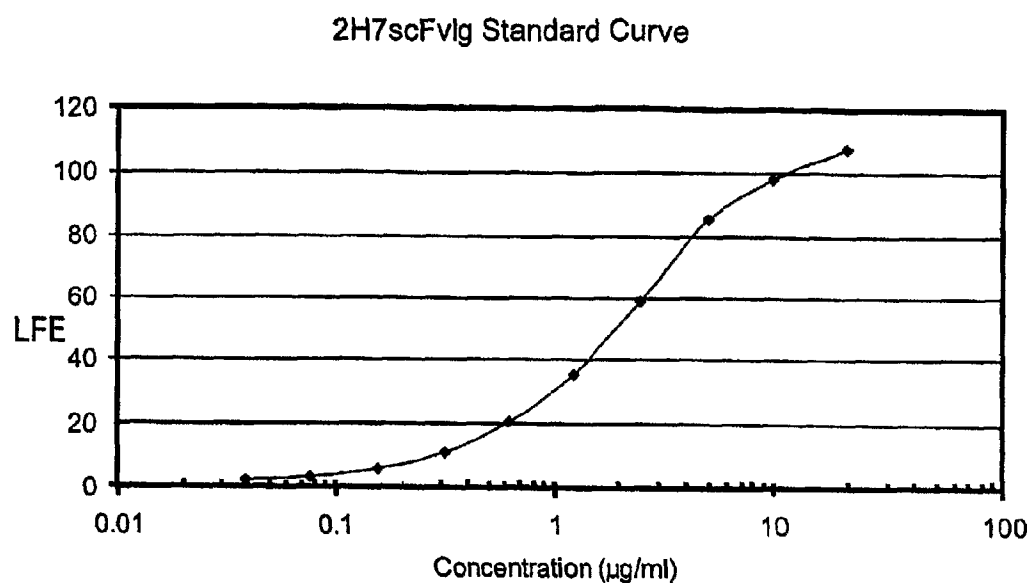
```
    P G K * S R
1501 CCGGGTAAAT GATCTAGA
```

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**FIG.2**



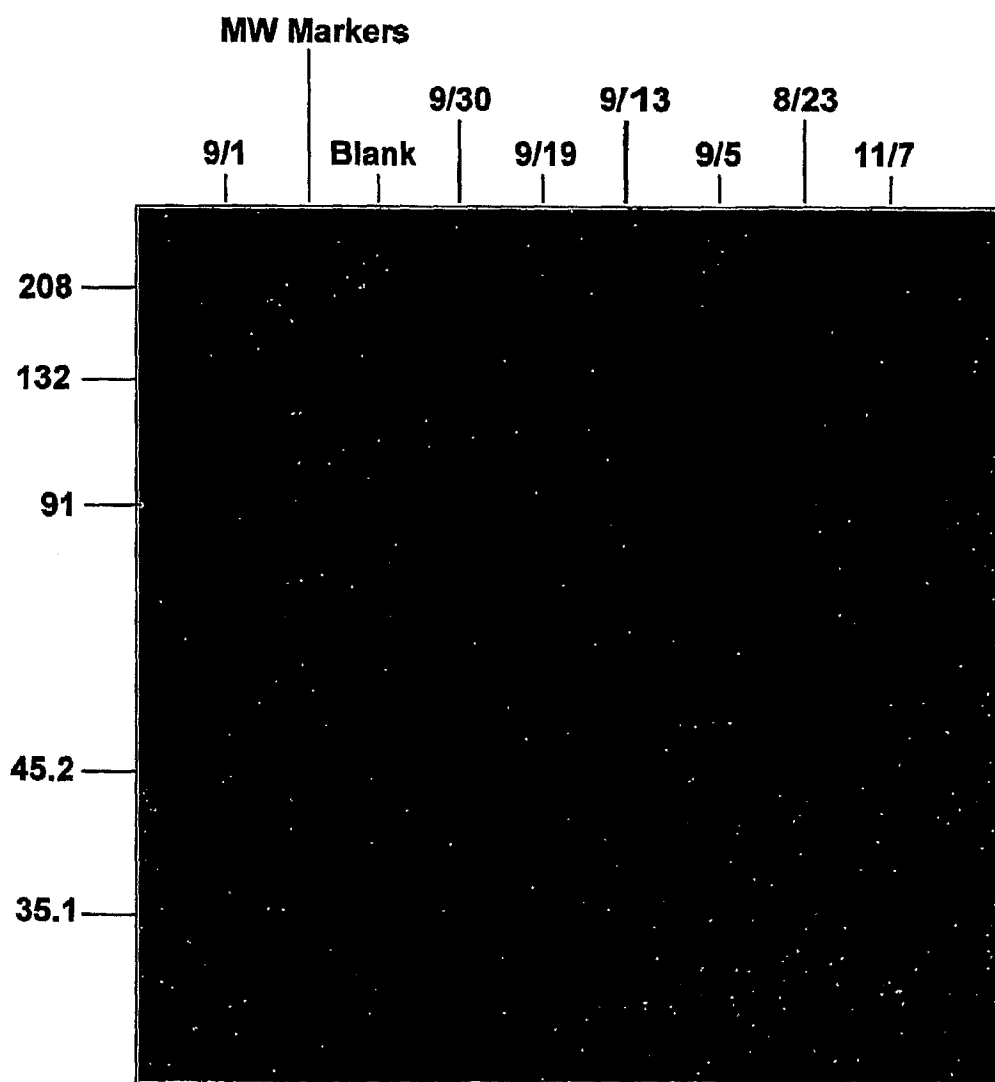
| Clone      | LFE @ 1:50 | Estimated Concentration<br>(µg/ml) |
|------------|------------|------------------------------------|
| D2         | 26.1       | 56                                 |
| IIIC6      | 25.7       | 55                                 |
| IVA3       | 28.6       | 61                                 |
| Spent bulk | 29.6       | 64                                 |

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**FIG.3**



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## FIG. 4A

**Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:**

| 2H7scFv-Ig Concentration | RAMOS | BJAB |
|--------------------------|-------|------|
| 20 µg/ml + complement    | 0.16  | 0.07 |
| 5 µg/ml + complement     | 0.2   | N.D. |
| 1.25 µg/ml + complement  | 0.32  | 0.1  |
| Complement alone         | 0.98  | 0.94 |

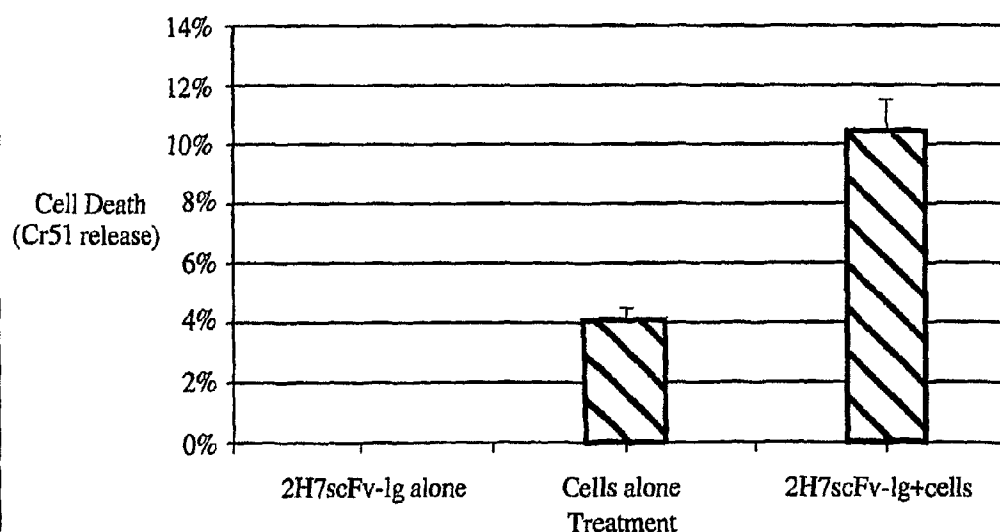
\*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

\*\*N.D. (not determined).

## FIG. 4B

**Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:**

**ADCC Activity of 2H7scFv-Ig on Ramos Cells**

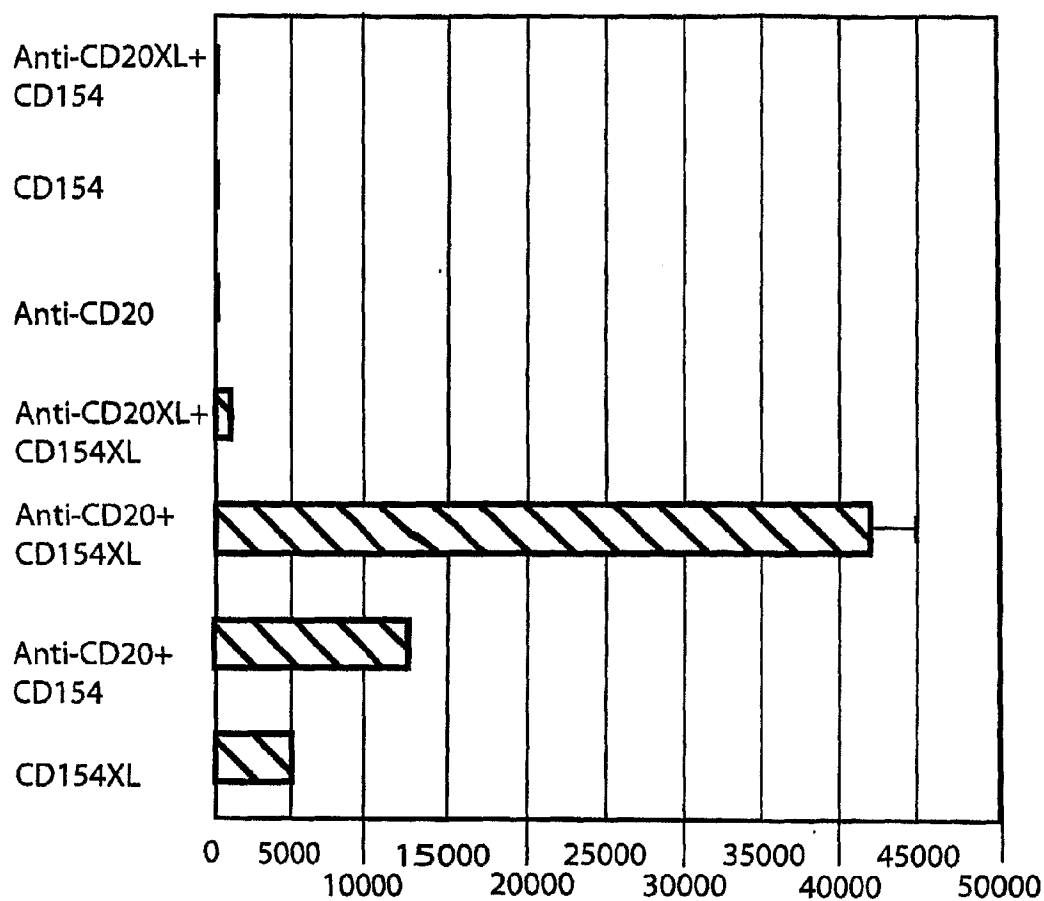


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**FIG. 5**

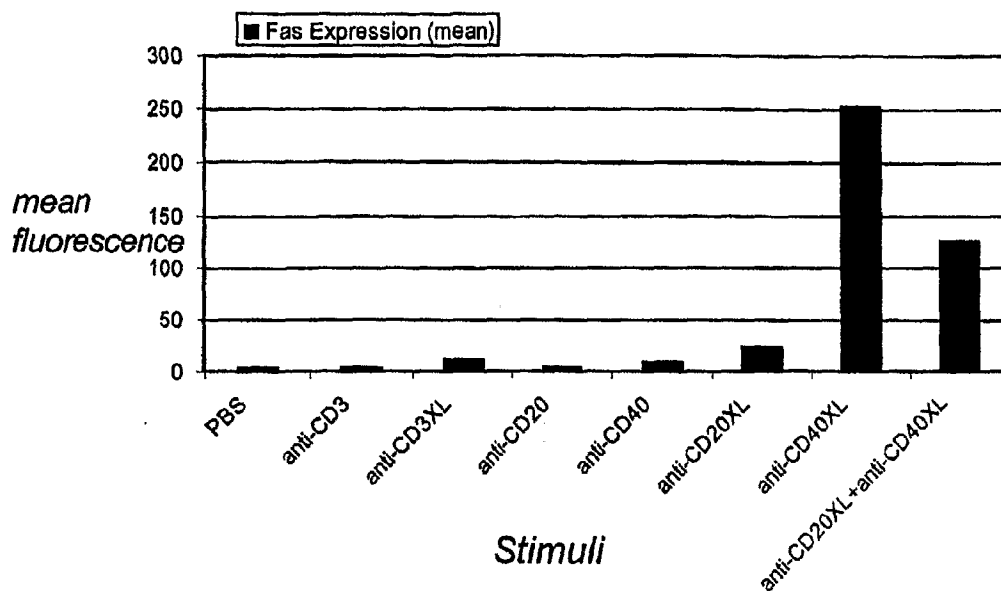


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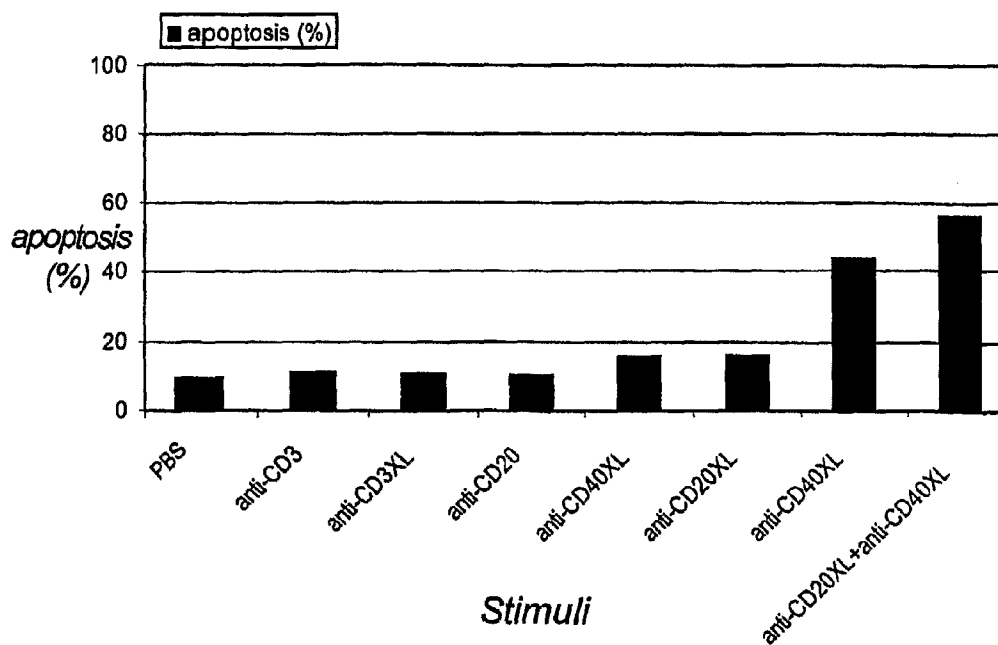
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**FIG.6A**



**FIG.6B**



**FIG. 7A**

HindIII      NcoI    2H7 V<sub>L</sub> Leader Peptide →

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**FIG.7B**

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 | G T G T T V T V S D P R R L D K I E D E  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E  
AGGAATCTTC ATGAAGATTT TGTATTTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K  
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G  
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

~~~~~

1021 D Q N P Q I A A H V I S E A S S K T T S
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTCGGAA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCGCGCAA

1321 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

~~~~~

1381 V F V N V T D P S Q V S H G T G F T S F
GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI

XbaI

~~~~~

~~~~~

1441 G L L K L E * * S R
GGCTTACTCA AACTCGAGTG ATAATCTAGA

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FIG. 7C**2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:**

```
HindIII      NcoI
~~~~~      ~~~~~2H7 VL Leader Peptide→
              M D F Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

              2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTGTCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

              BamHI
              ~~~~~
      Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT TATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

              (Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

              2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

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FIG.7D

human CD154/amino acid 108 →

```

                                Bcl/Bam hybrid site          BclI
      G T G T T V T V S D P E N S F E M Q K G
781  GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      -----
      D Q N P Q I A A H V I S E A S S K T T S
841  GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

      V L Q W A E K G Y Y T M S N N L V T L E
901  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

      N G K Q L T V K R Q G L Y Y I Y A Q V T
961  AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                HindIII
                                -----
      F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

      S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

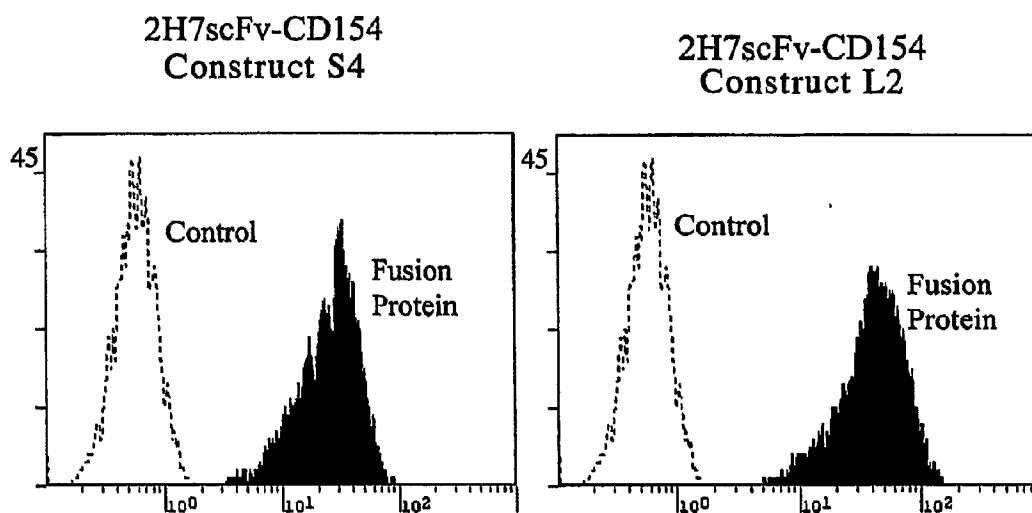
      P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                NcoI
                                -----
      V F V N V T D P S Q V S H G T G F T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                                XhoI          XbaI
                                -----
      G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA
  
```

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FIG. 8



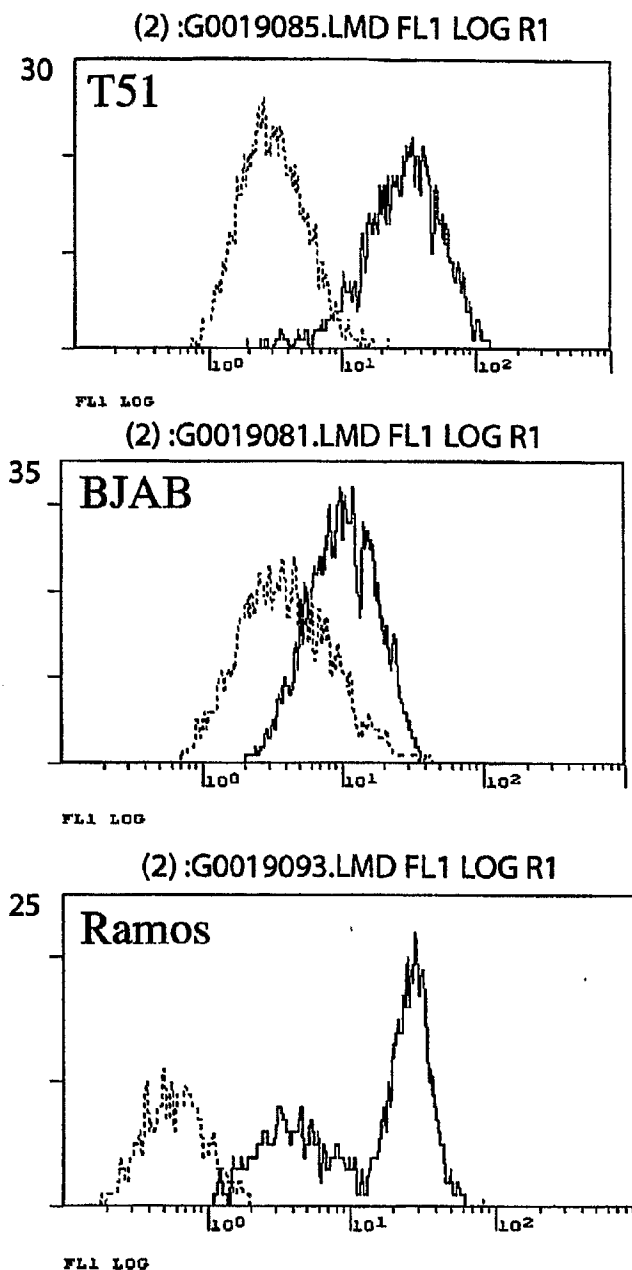
CD20 CHO cell targets + (control or fusion protein)
+ Biotin-CD40Ig + PE-SA

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FIG.9



.....control supernatant 2H7scFv-CD154 supernatant

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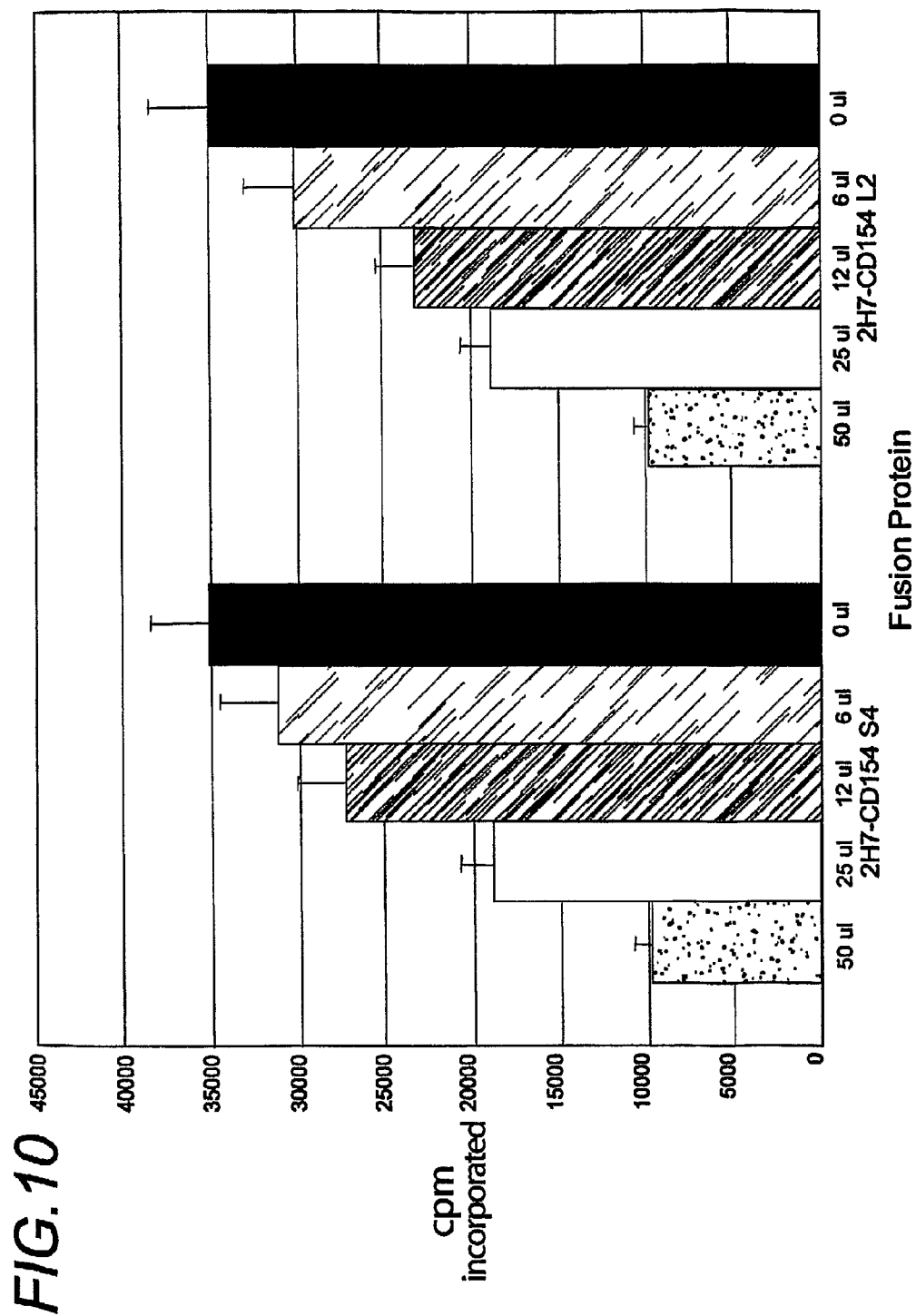
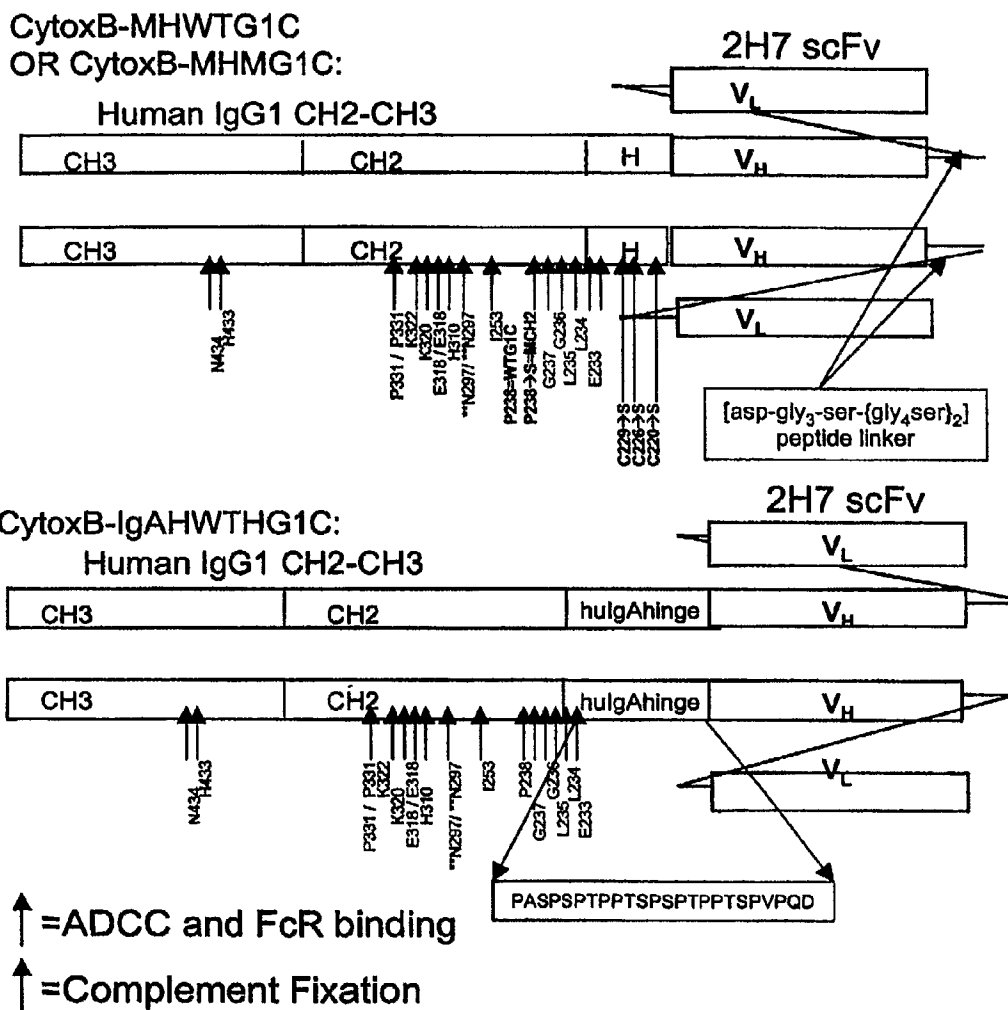


FIG. 11

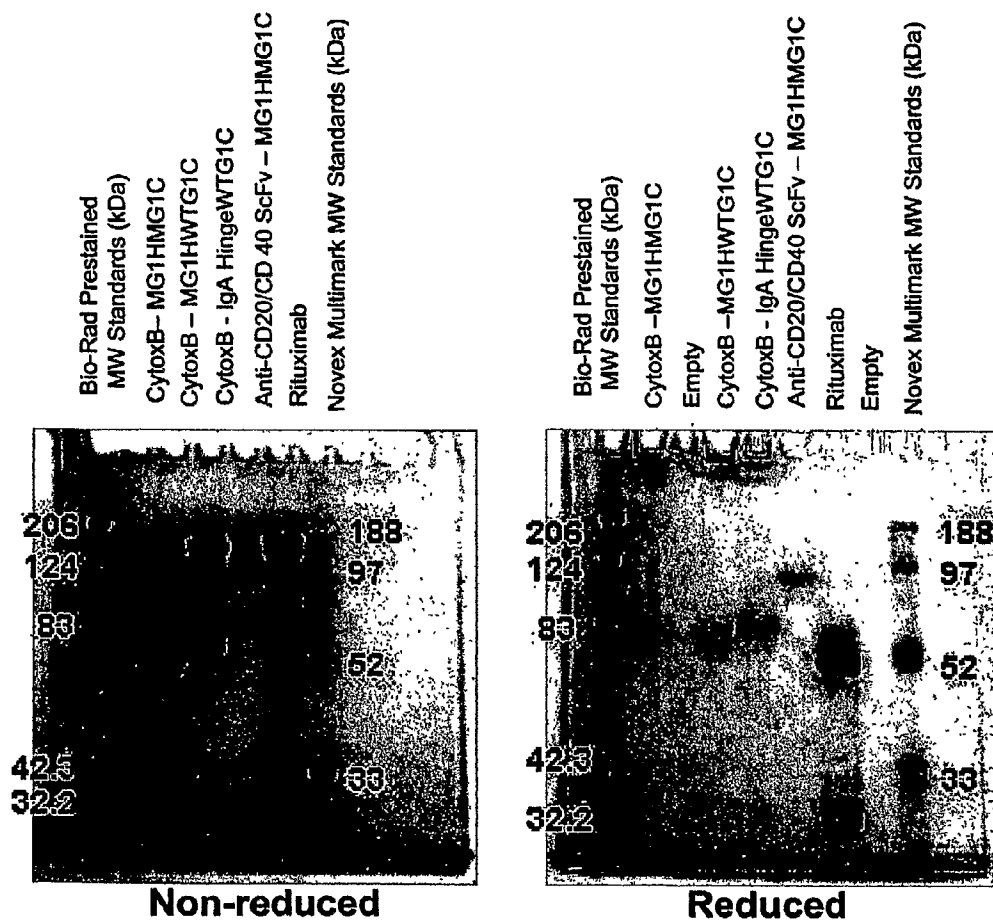


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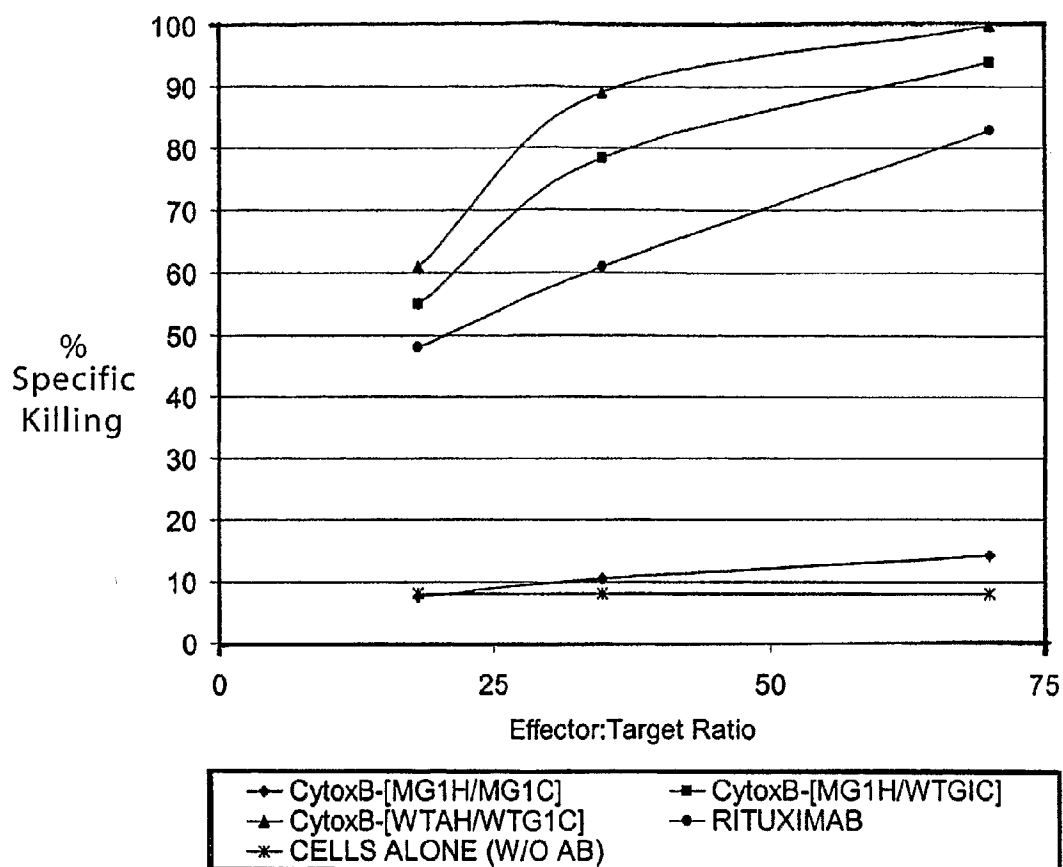
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FIG. 12



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FIG. 13

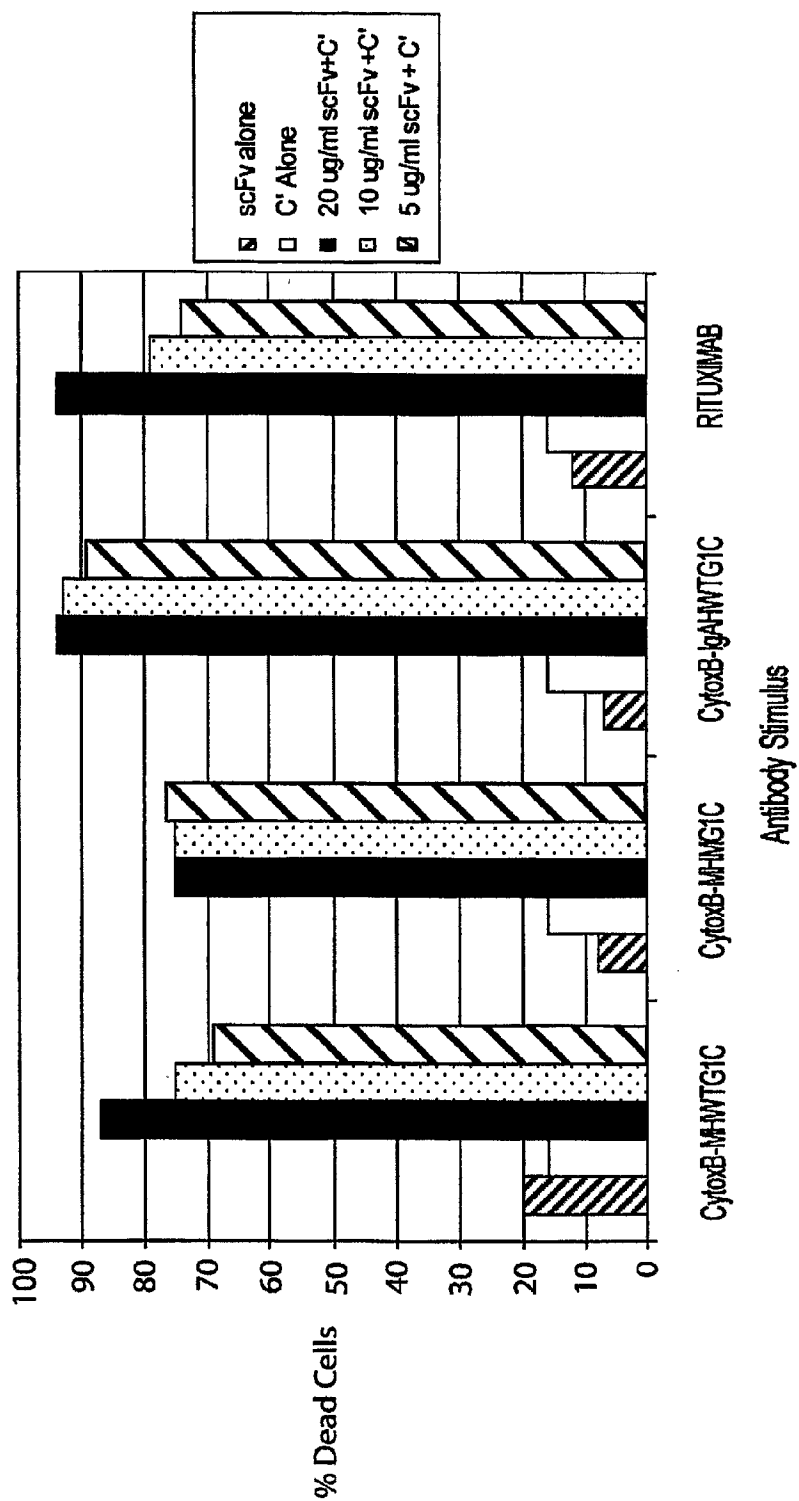


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FIG.14

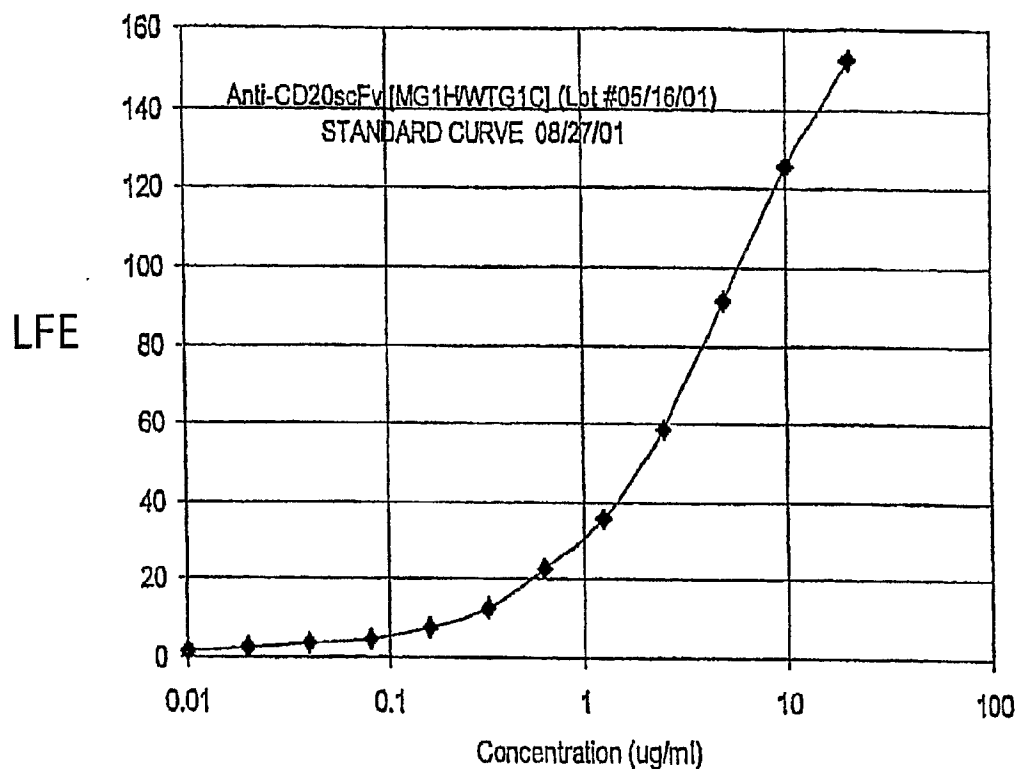


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FIG. 15

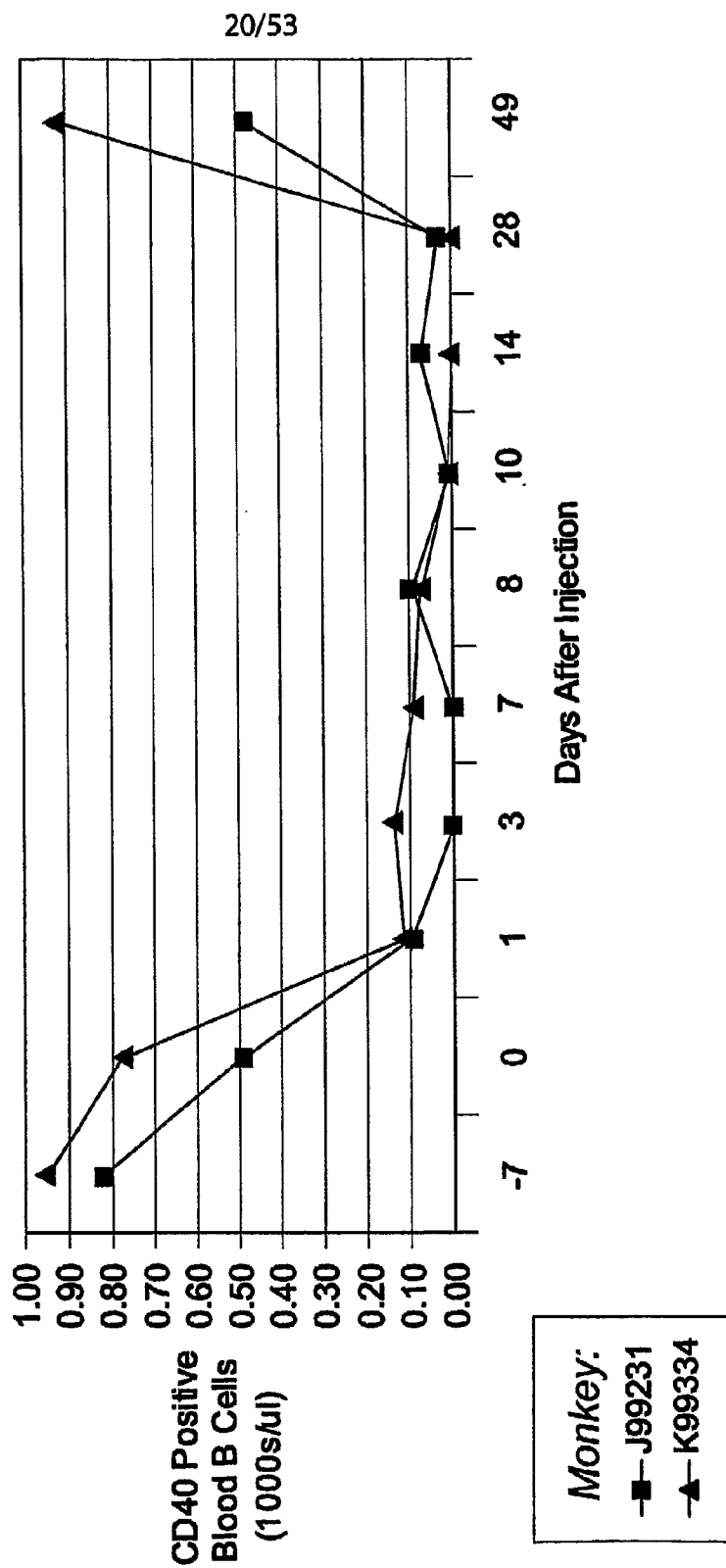


Day	Monkey J99231		Monkey K99334	
	LFE(1:40)	Concentration ($\mu\text{g/mL}$)	LFE(1:40)	Concentration ($\mu\text{g/mL}$)
Injection \rightarrow -7	2.41	<0.6 $\mu\text{g/mL}$	1.51	<0.4 $\mu\text{g/mL}$
0	2.22	<0.6 $\mu\text{g/mL}$	1.63	<0.4 $\mu\text{g/mL}$
1	73.8	220 $\mu\text{g/mL}$	44.4	100 $\mu\text{g/mL}$
Injection \rightarrow 3	20.0	28 $\mu\text{g/mL}$	40.2	80 $\mu\text{g/mL}$
7	15.6	24 $\mu\text{g/mL}$	15.7	24 $\mu\text{g/mL}$
8	39.1	80 $\mu\text{g/mL}$	42.6	92 $\mu\text{g/mL}$
10	11.5	18 $\mu\text{g/mL}$	2.74	1.2 $\mu\text{g/mL}$
14	2.05	0.6 mg/mL	1.96	0.6 $\mu\text{g/mL}$

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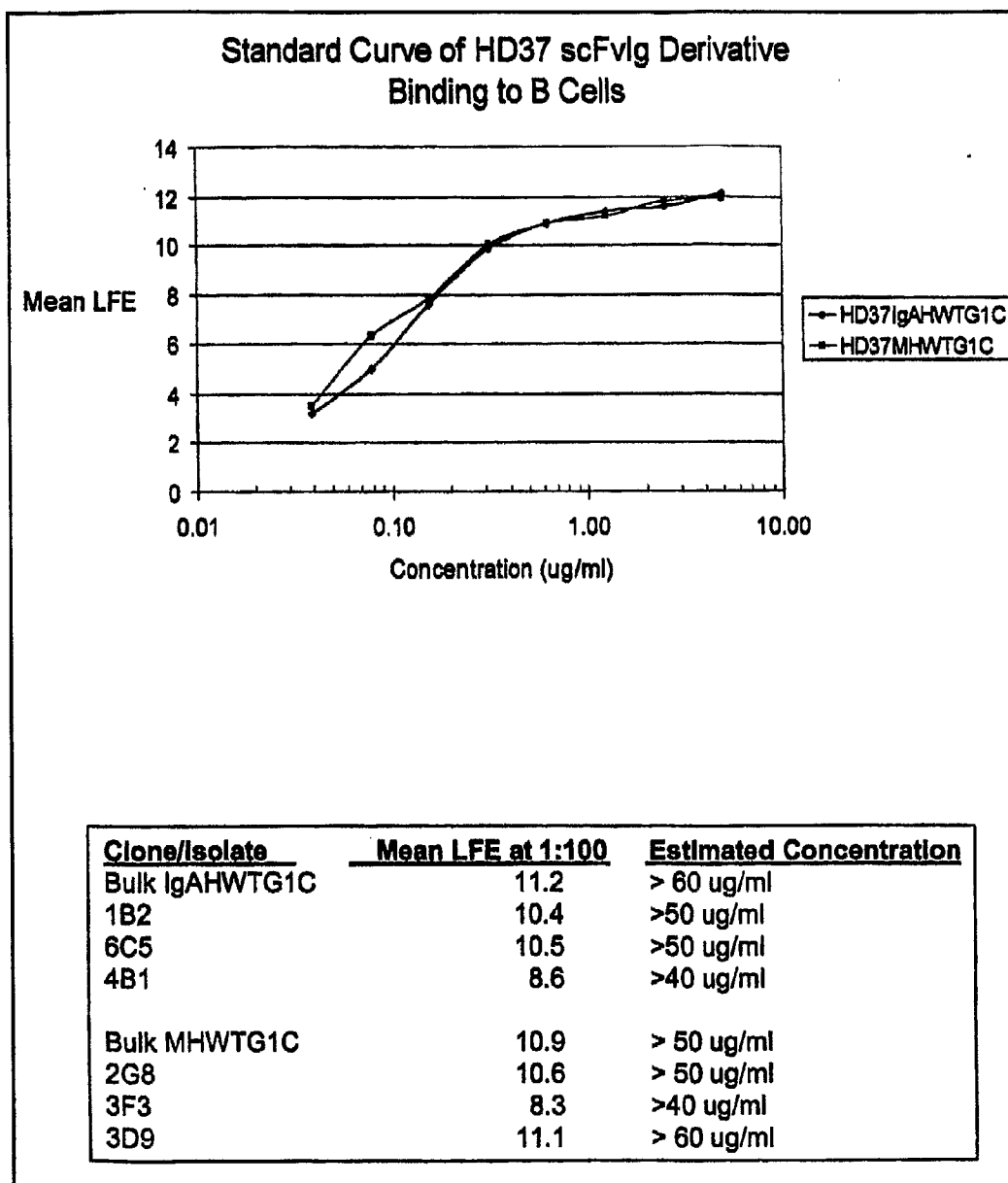
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FIG. 16



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FIG.17

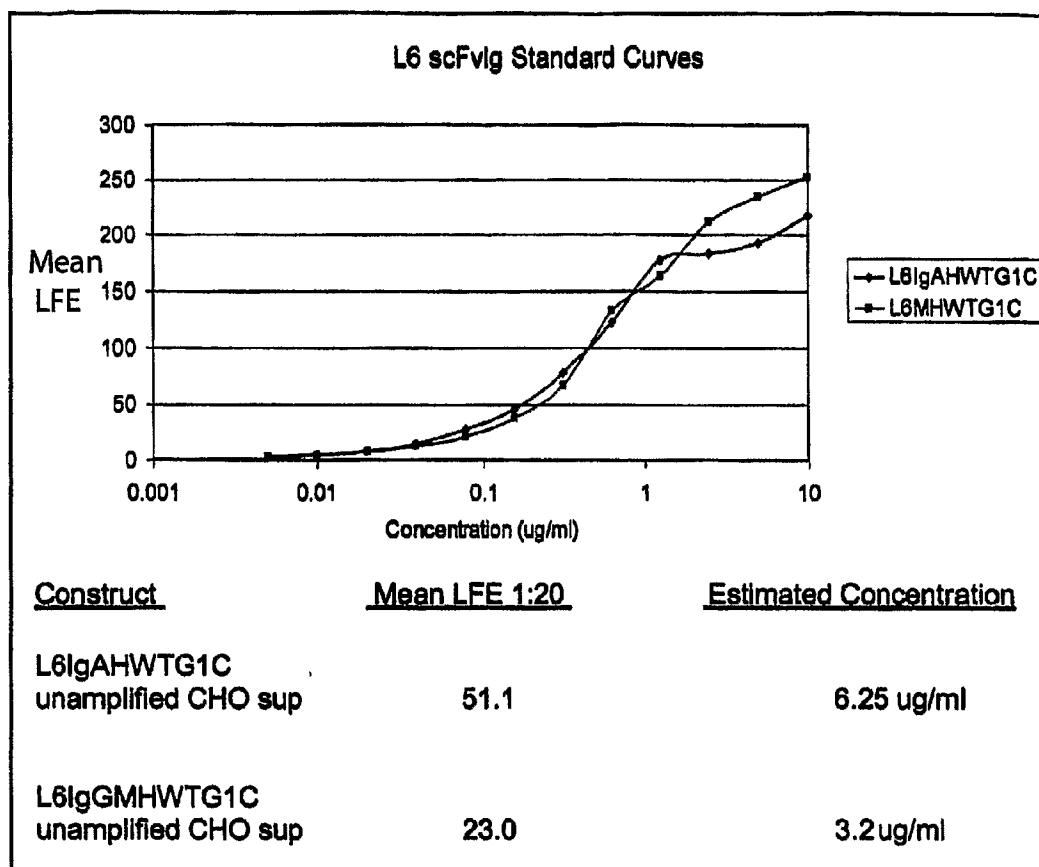


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FIG. 18



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FIG. 19A

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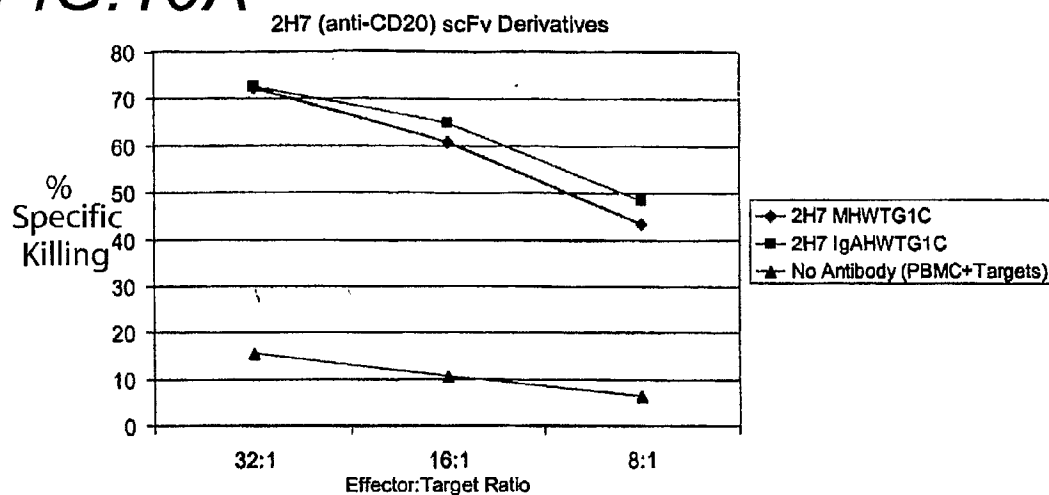


FIG. 19B

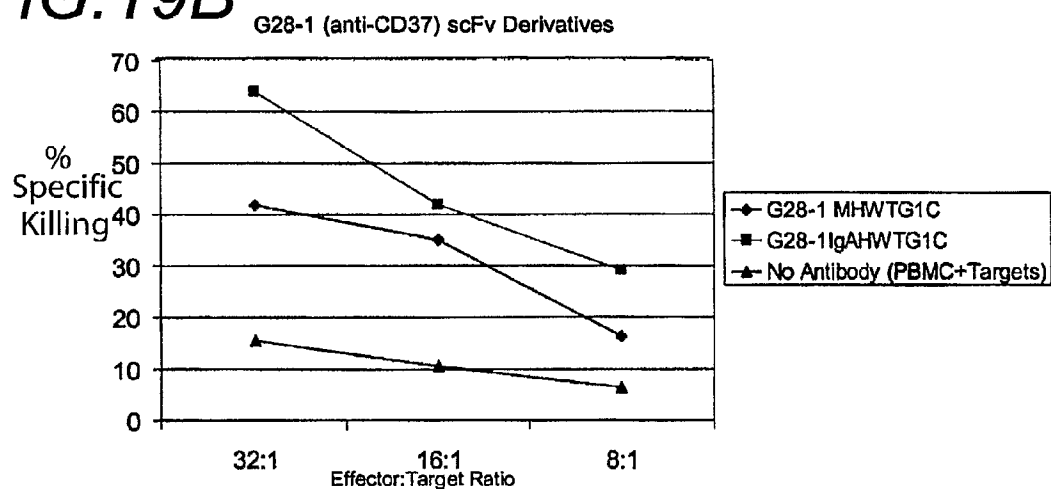
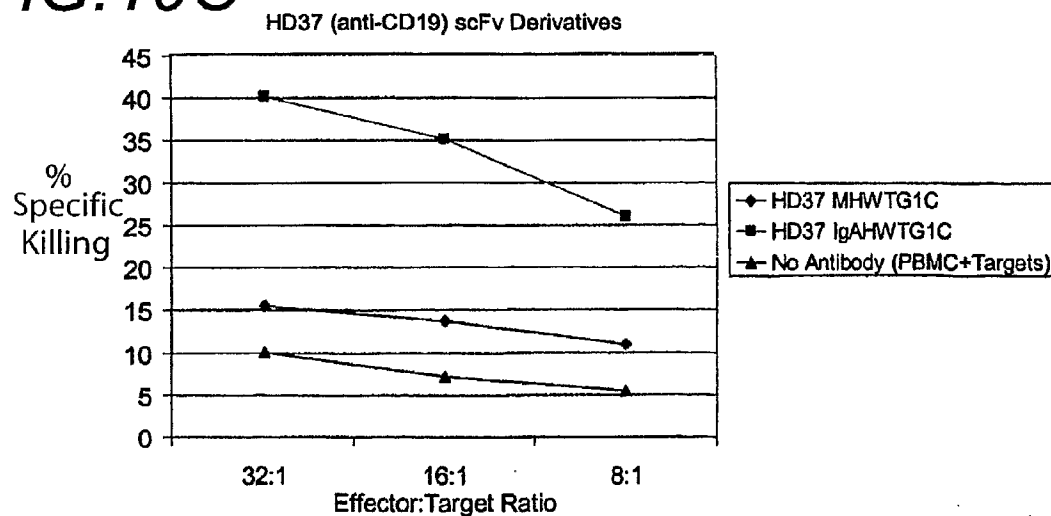


FIG. 19C

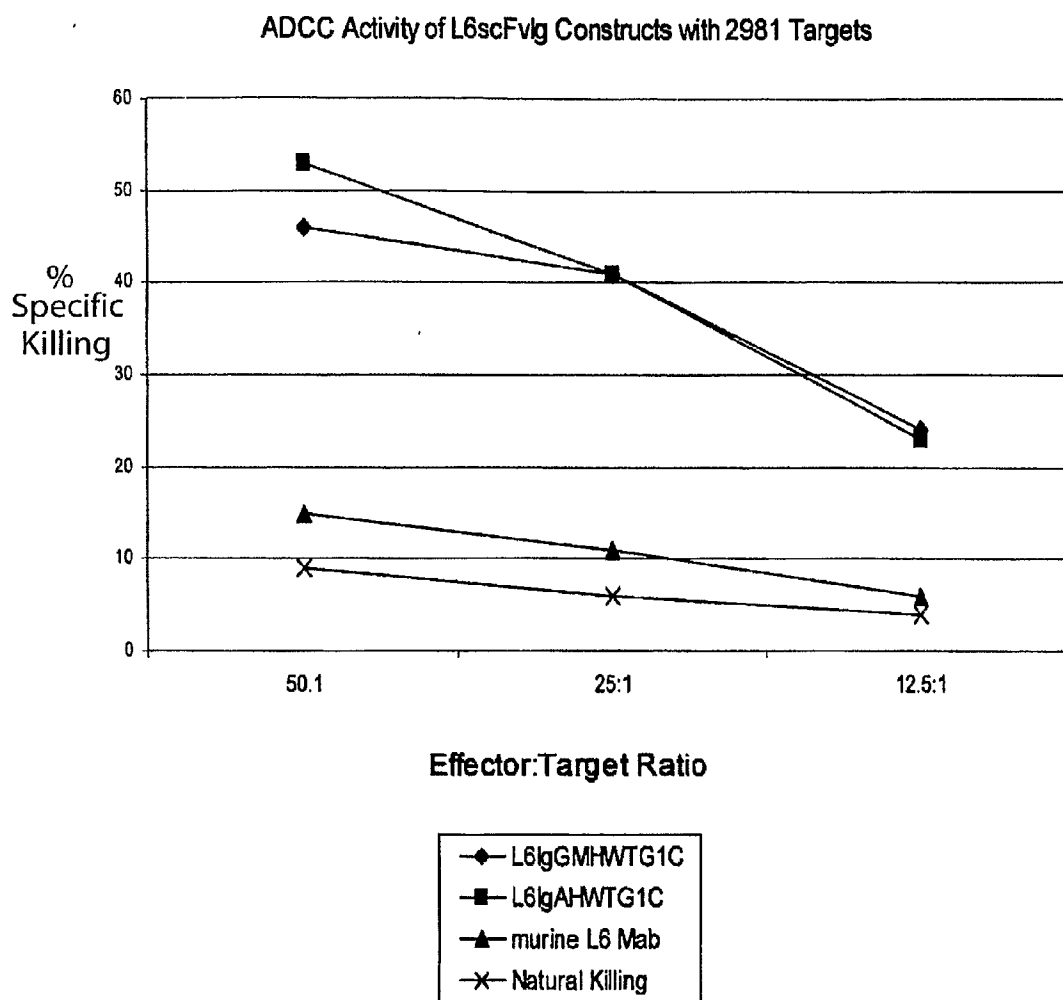


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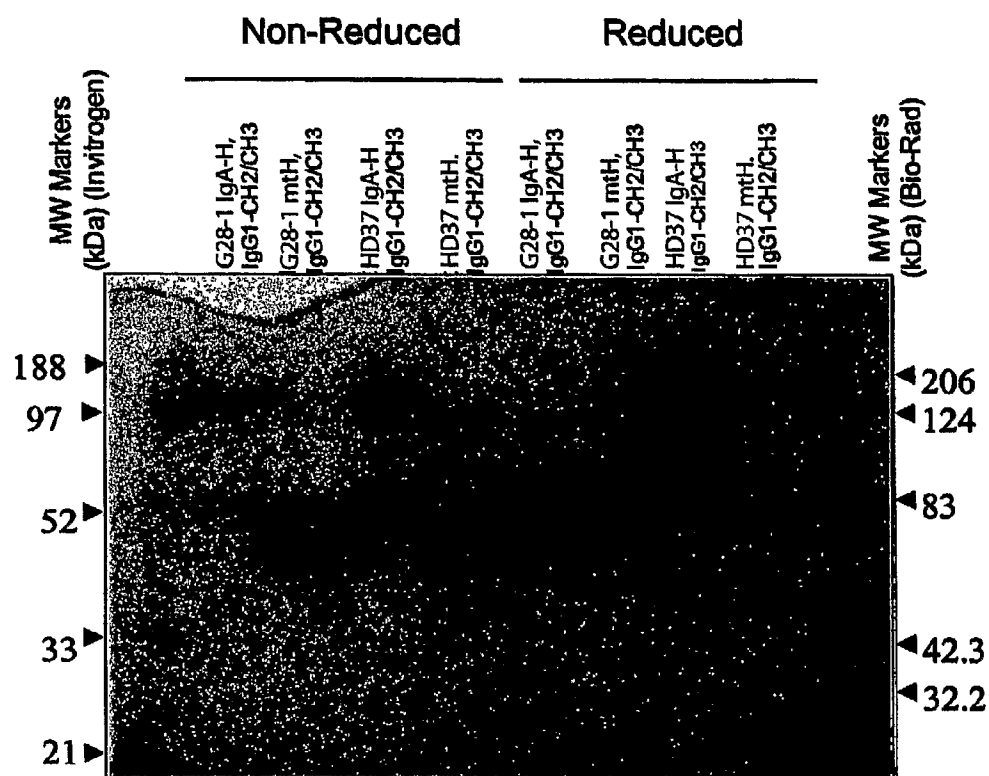
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FIG.20



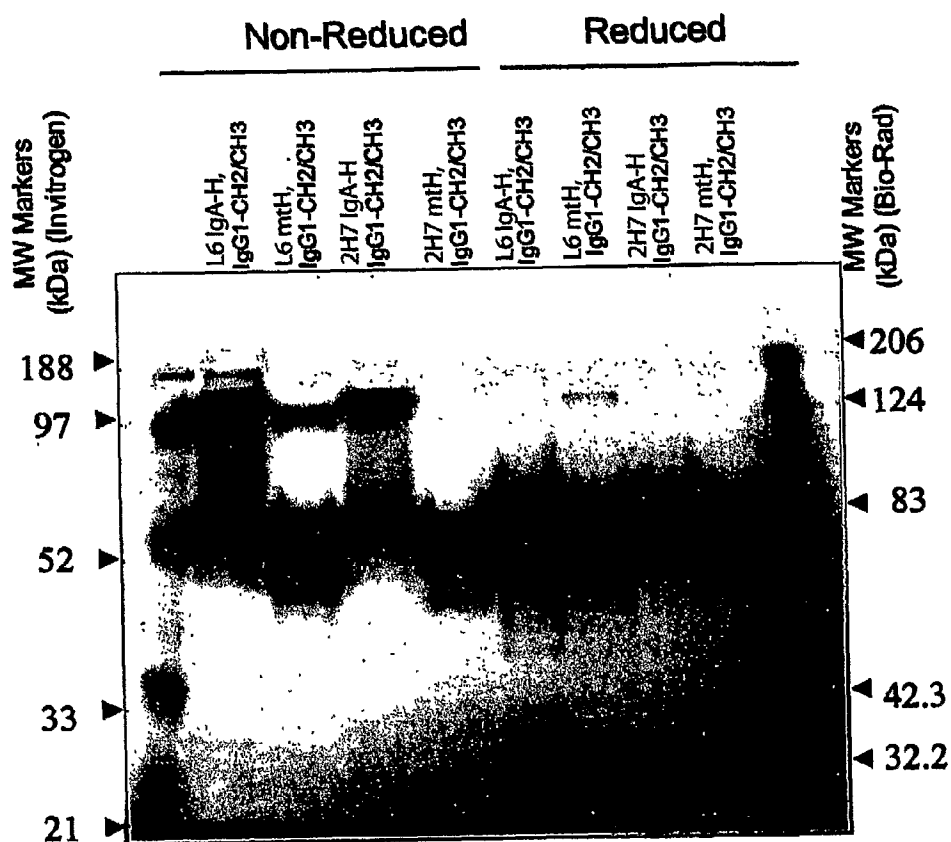
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FIG.21



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FIG.22



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FIG.23

	Hinge	CH2
Human IgG1:	DOEPKSCDKT-----HTCPPC	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
Llama IgG2:	DOEPKTPKQPQPPQPNPTTESKCPKC	PAPELLGGPSVFLFPPKPKDVLISIGRPEVTCVVVDVGQEDPEVSFNWYIDG
Llama IgG1:	--EPHGG-----CTCPQC	PAPELLGGPSVFLFPPKPKDVLISIGRPEVTCVVVDVGKEDPEVNFNWIIDG
Llama IgG3:	--AHHSEDP-----SKCPKC	PGPELLGGPTVFIFFPKAKDVLISITRKPEVTCVLTWVTKTLRSSSSWSVDD
CH3		
VEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYITLPPSRDELTKNQVSLT		
TAEVRASTRPKEEQFNSTYRVVSVLP IQHQDWLTGKEFKCKVNNKALPAPIEKTIISKAKGQTPREPQVYITLAPHREELAKDTVSVT		
VEVRTANTKPKKEEQFNSTYRVVSVLP IQHQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYITLAPHREELAKDTVSVT		
TEVHTAETKPKKEEQFNSTYRVVSVLP IQHQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYITLAPHREELAKDTVSVT		
CH2		
CLVKGFFPSDIAVWESNGQPEN--NYKTTPPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		
CLVKGFFPPDINVEWQRNGQPESEGTATTPPQLDNDGTFFLYSKXKASVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGK		
CLVKGFFPADINVEWQRNGQPESEGTANTPPQLDNDGTFFLYSLRSLVGKNTWQGETLTGVVMHEALHNHYTQKSITQSSGK		
CLVKGFFFPADINVEWQRNGQPESEGTANTPPQLDNDGTFFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHYTQKSITQSSGK		

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FIG.24

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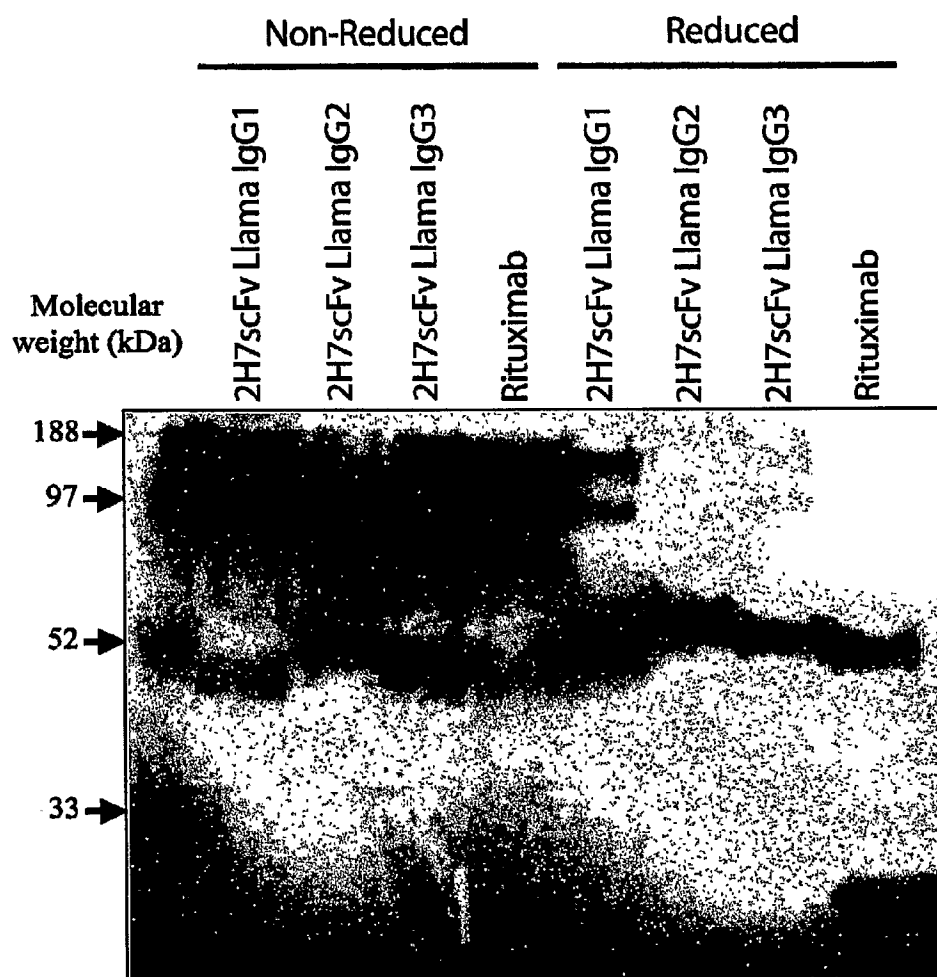
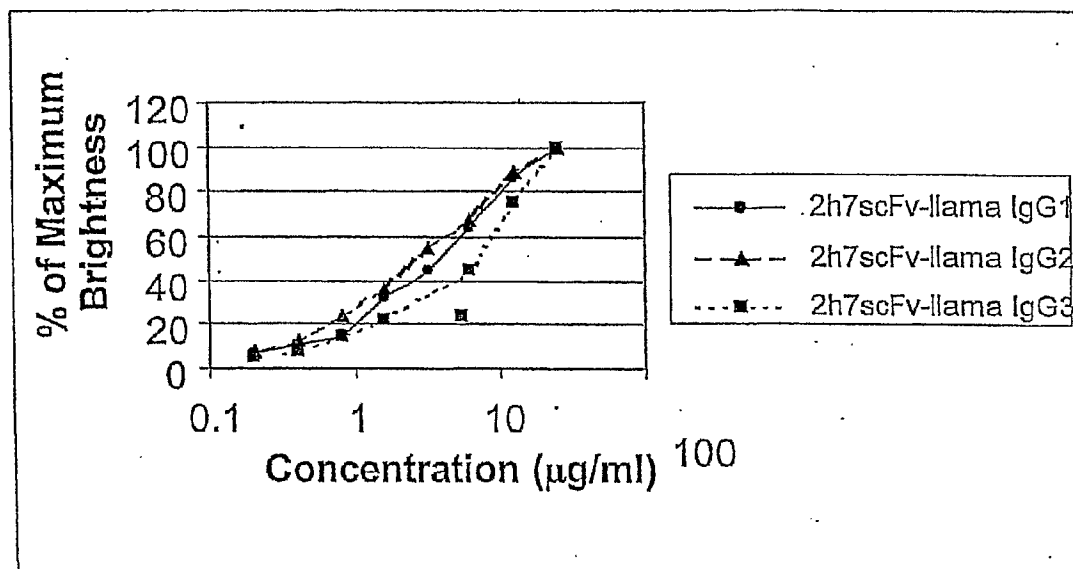
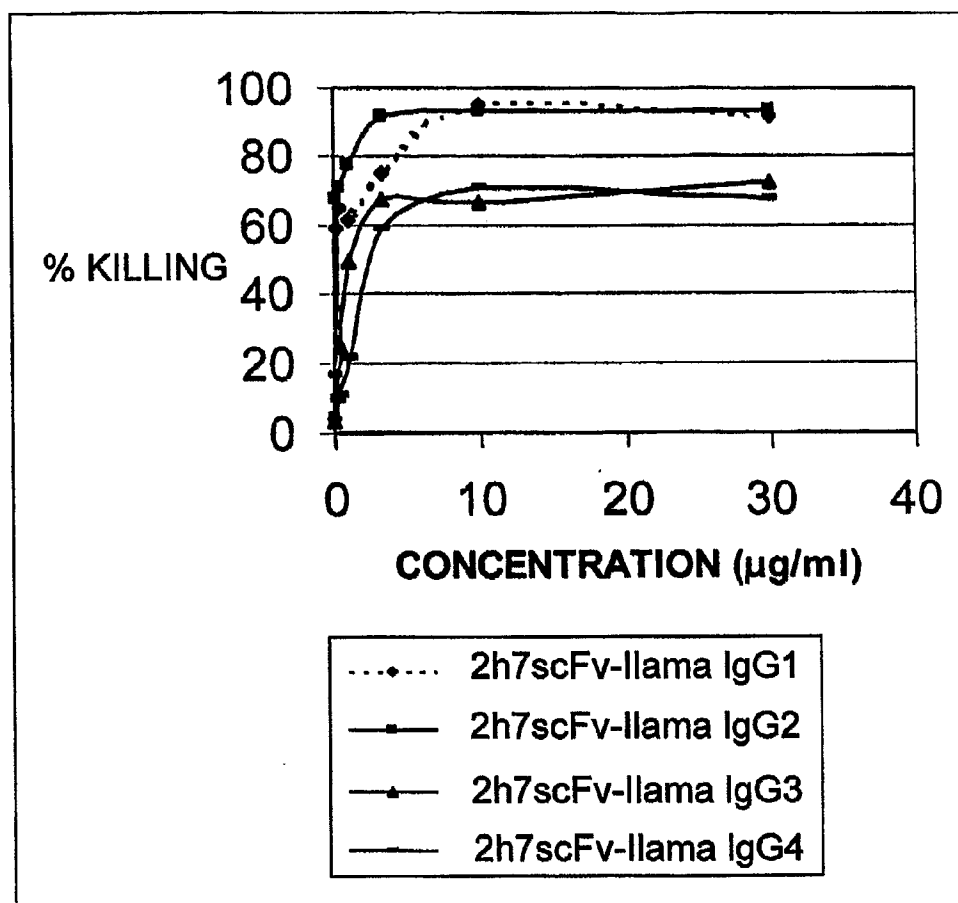


Figure 25



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FIG.26

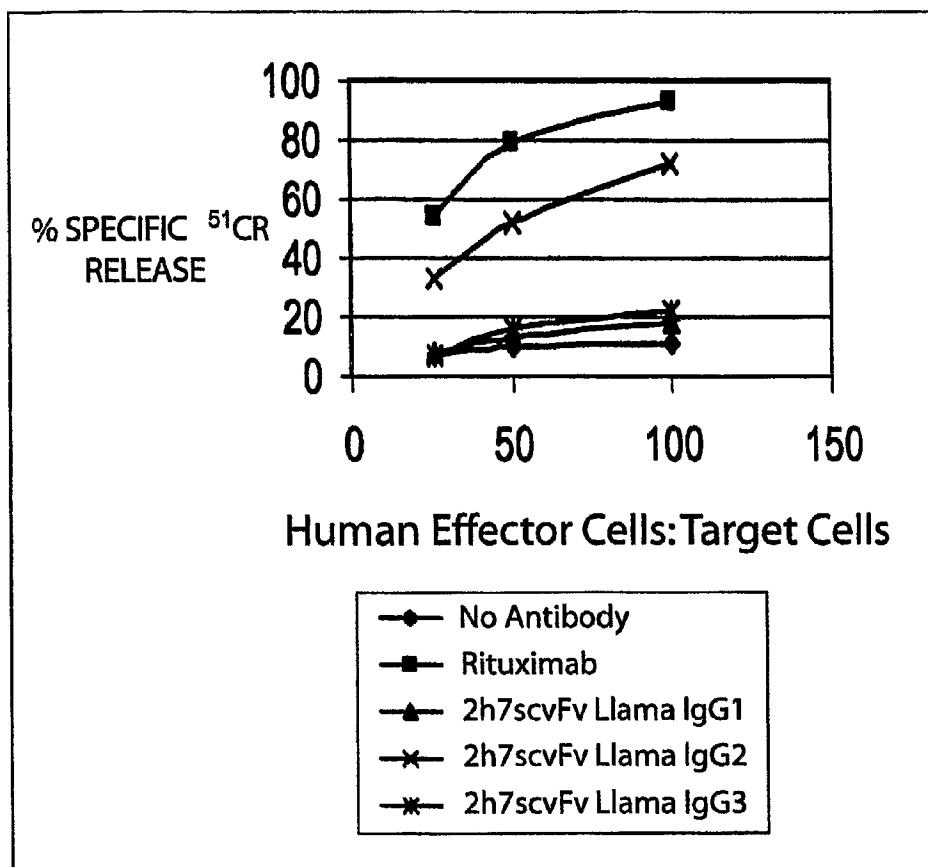


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FIG.27



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FIG.28

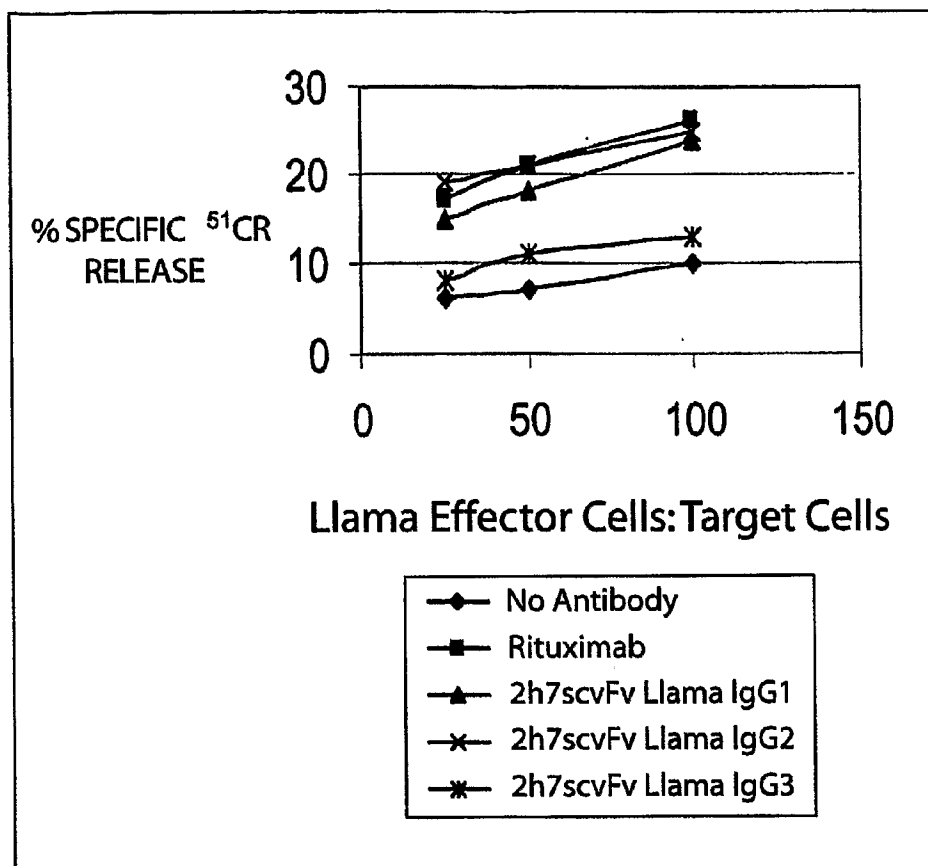
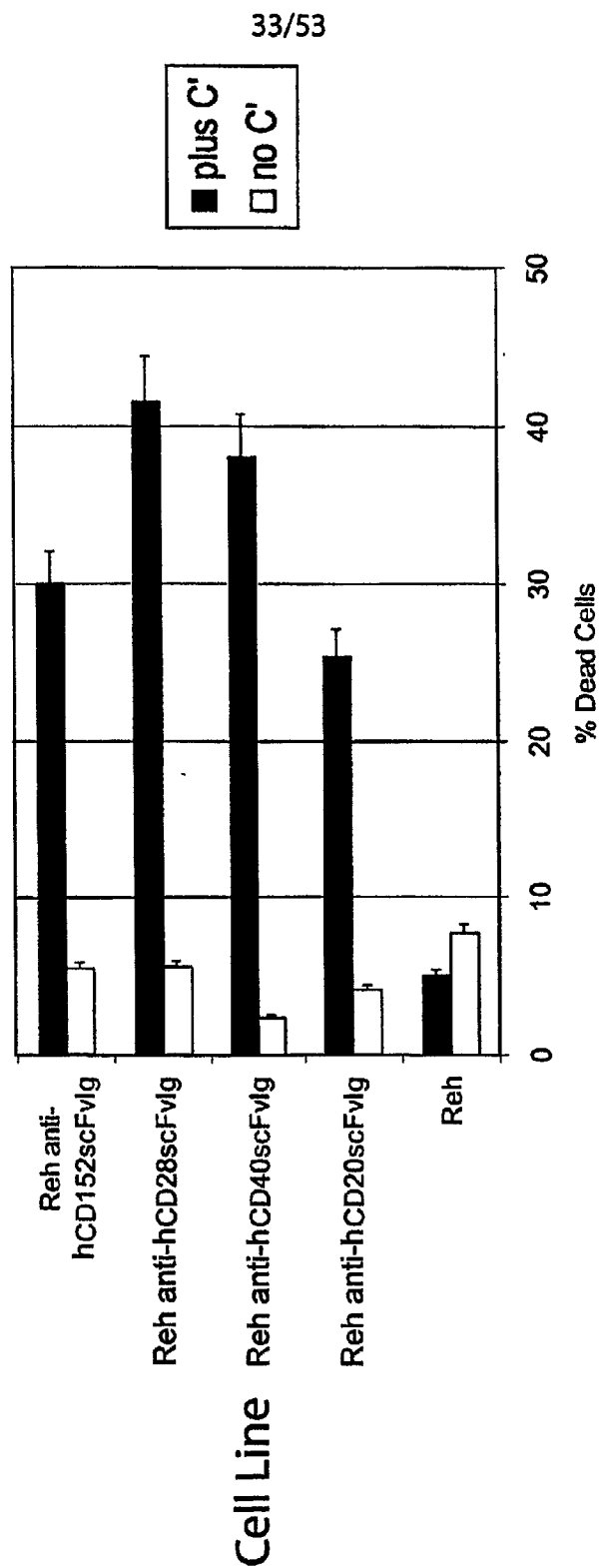
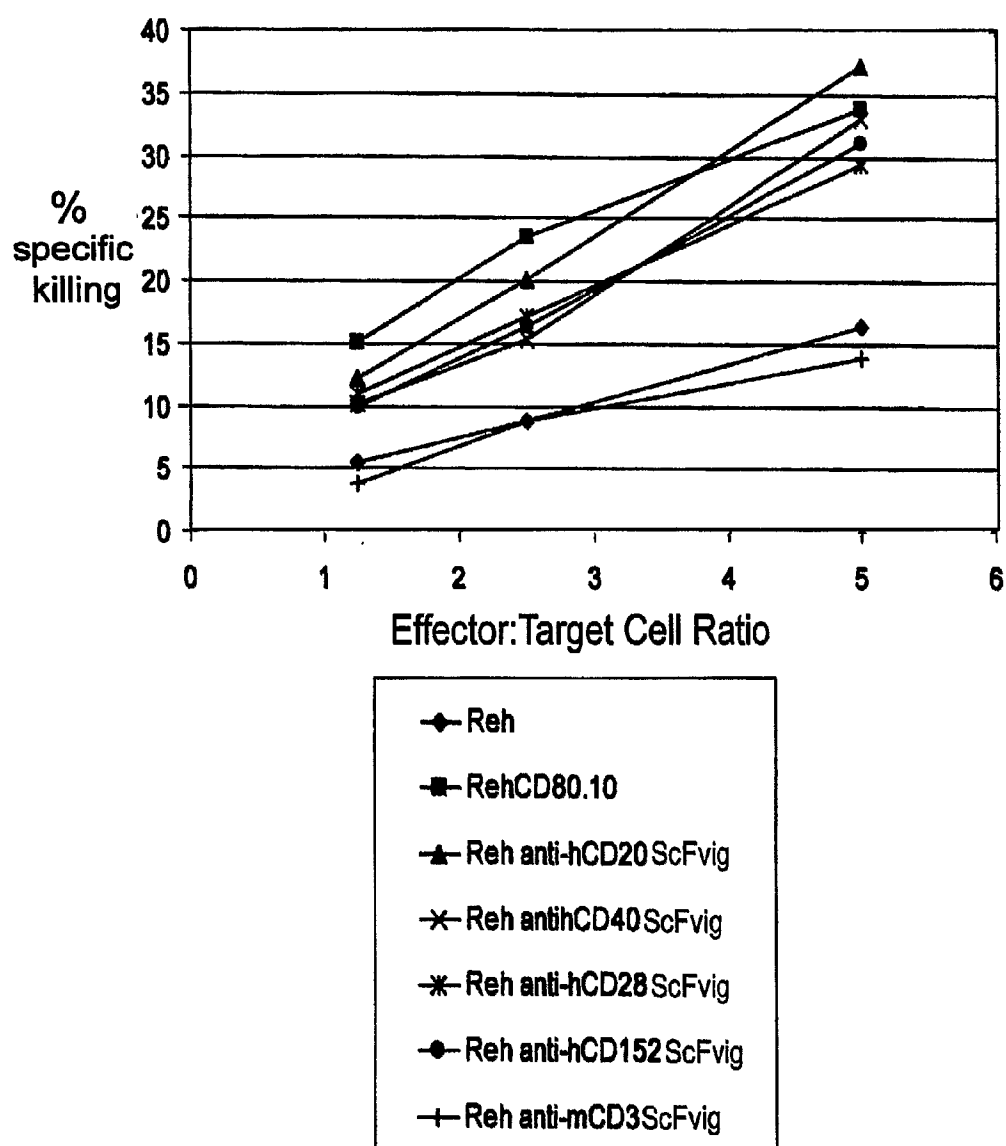


FIG. 29



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FIG.30



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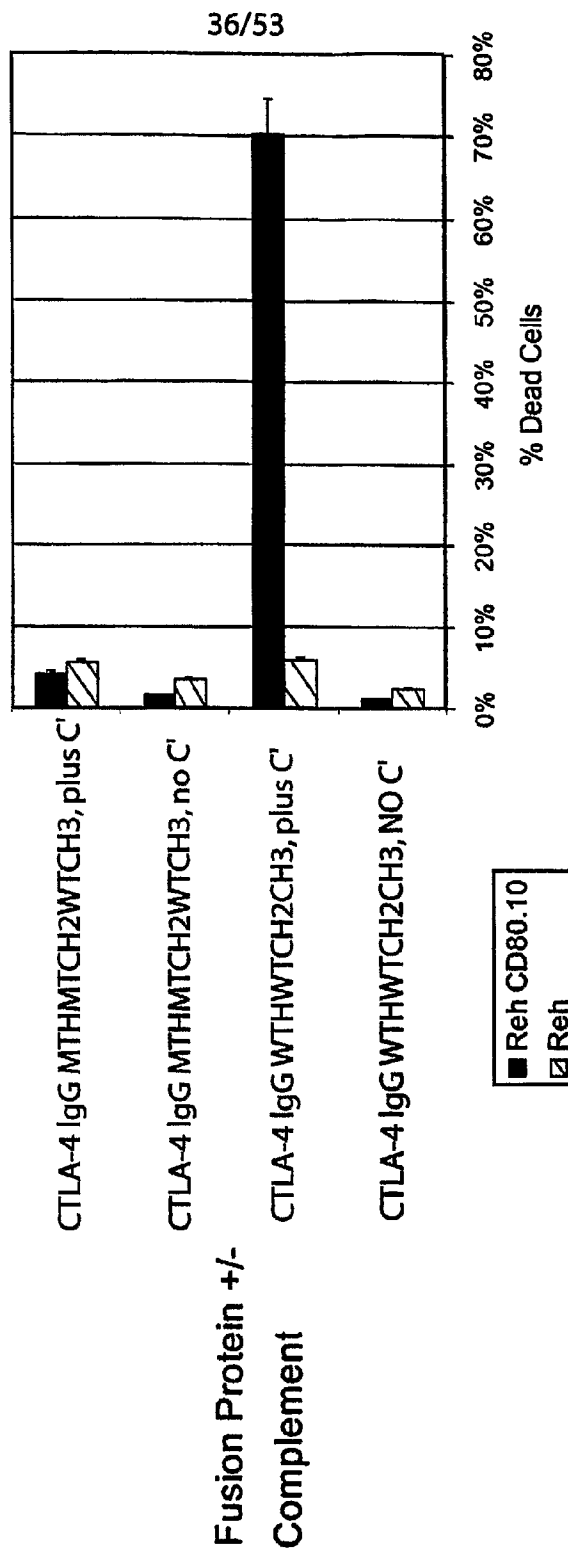
FIG. 31

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence	SEQ ID NO:
IgG WTH (CCC) WTCH2CH3	IgG1 WT Hinge (CCC)	Wild Type CH2	Wild Type CH3	
IgG MTH (SSS) WTCH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
VH SER 11 IgG MTH (SSS) WTCH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SSC) WTCH2CH3	IgG1 Mutant Hinge (SSC)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SCS) WTCH2CH3	IgG1 Mutant Hinge (SCS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (CSS) WTCH2CH3	IgG1 Mutant Hinge (CSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG MTH(SSS) MTCH2WTCH3	IgG1 Mutant Hinge (SSS)	Mutant CH2 (IgG1) Pro ? Ser 238	Wild type CH3 (IgG1)	
IgAH IgGWTCH2CH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgAH IgACH2CH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)	
IgAH IgA-T4	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) (deletion of 4 amino acids at carboxy terminus)	

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FIG.32



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FIG.33A

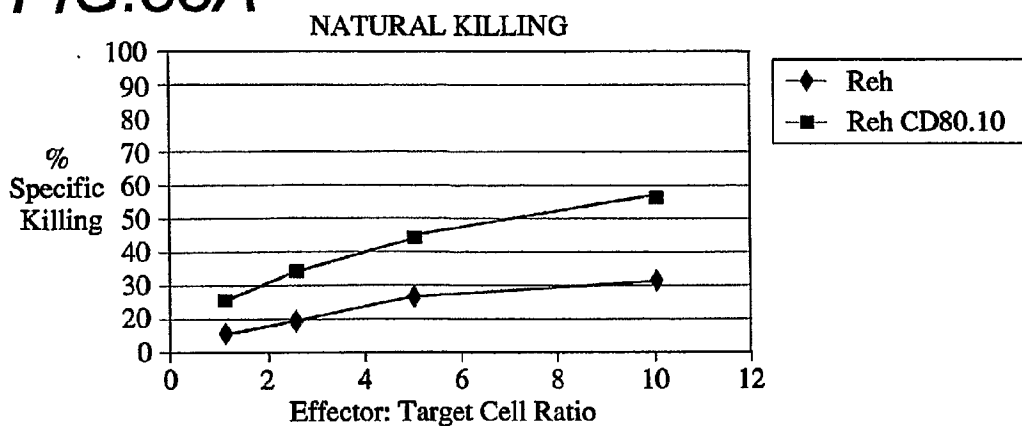


FIG.33B

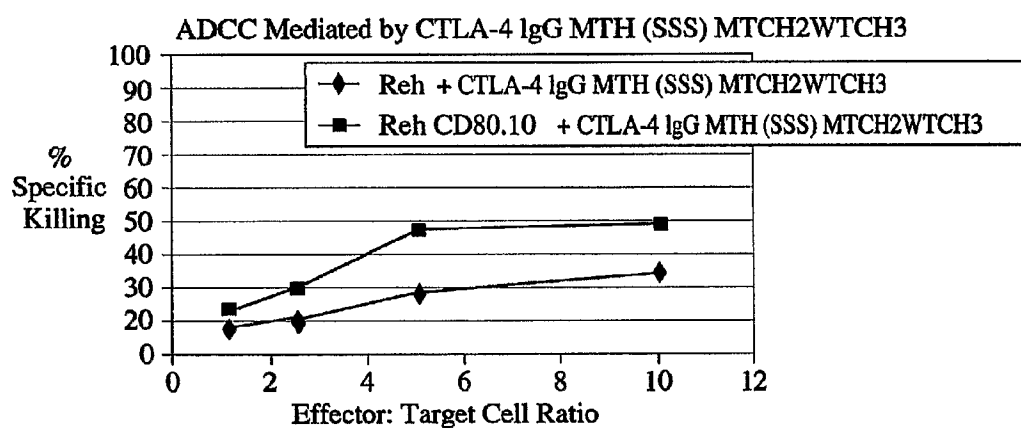
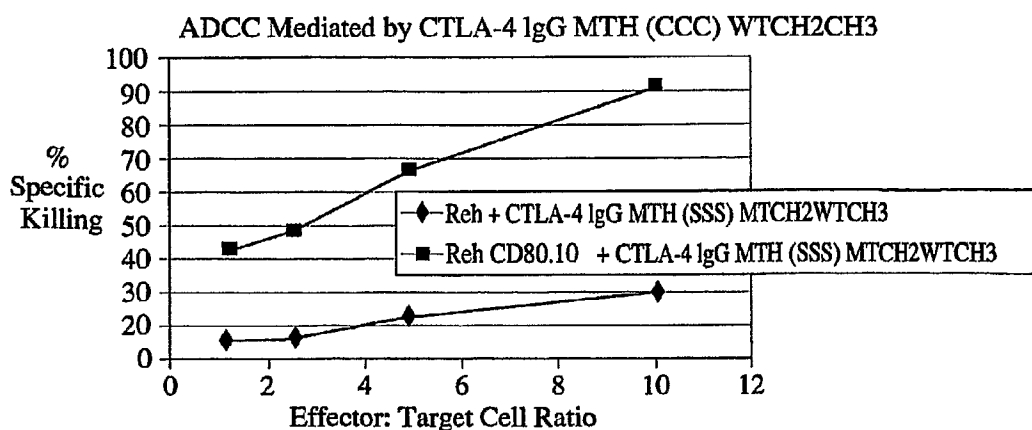
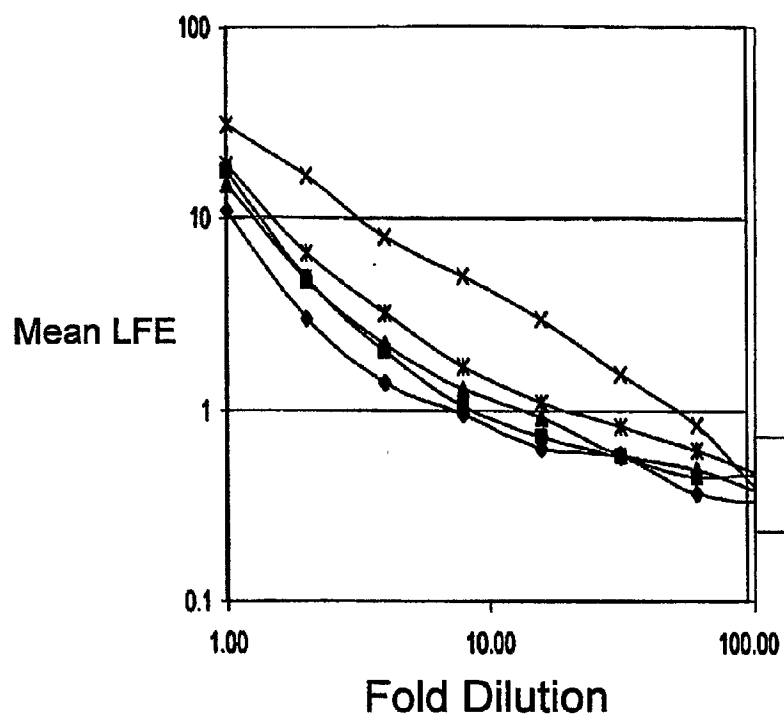


FIG.33C



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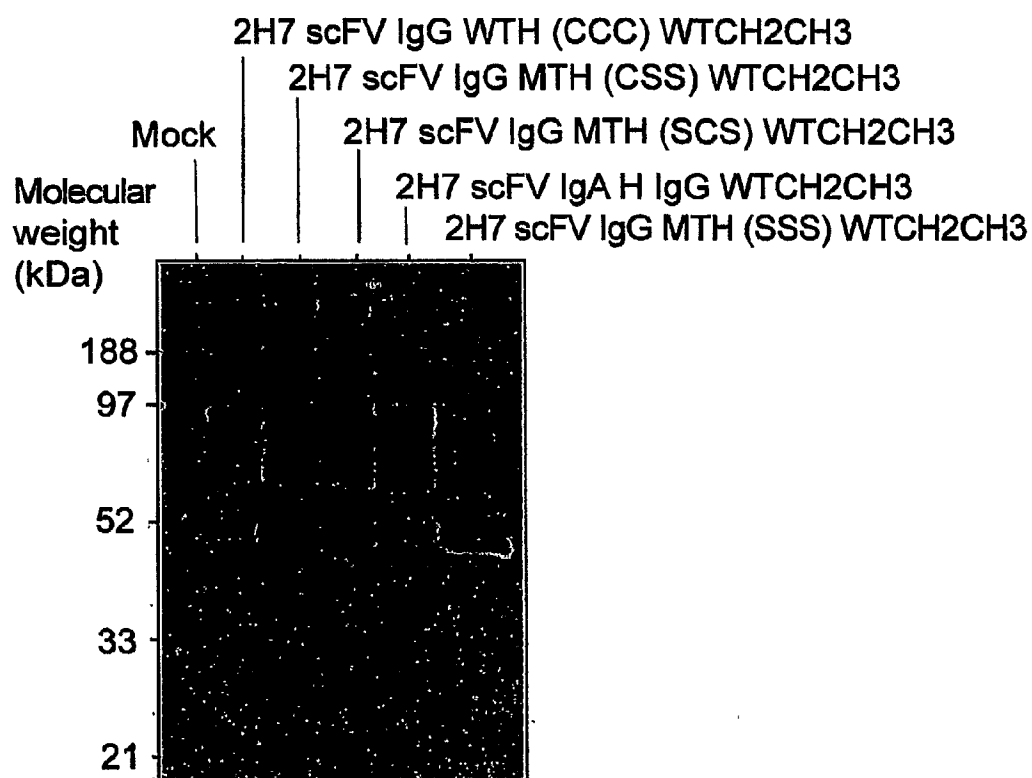
FIG.34



- ◆ 2H7 scFv WTH (CCC) WTCH2CH3
- 2H7 scFv MTH (CSS) WTCH2CH3
- ▲ 2H7 scFv MTH (SCS) WTCH2CH3
- ✱ 2H7 scFv MTH (SSC) WTCH2CH3
- ✱ 2H7 scFv VH11SER WTH (CCC) WTCH2CH3

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FIG.35

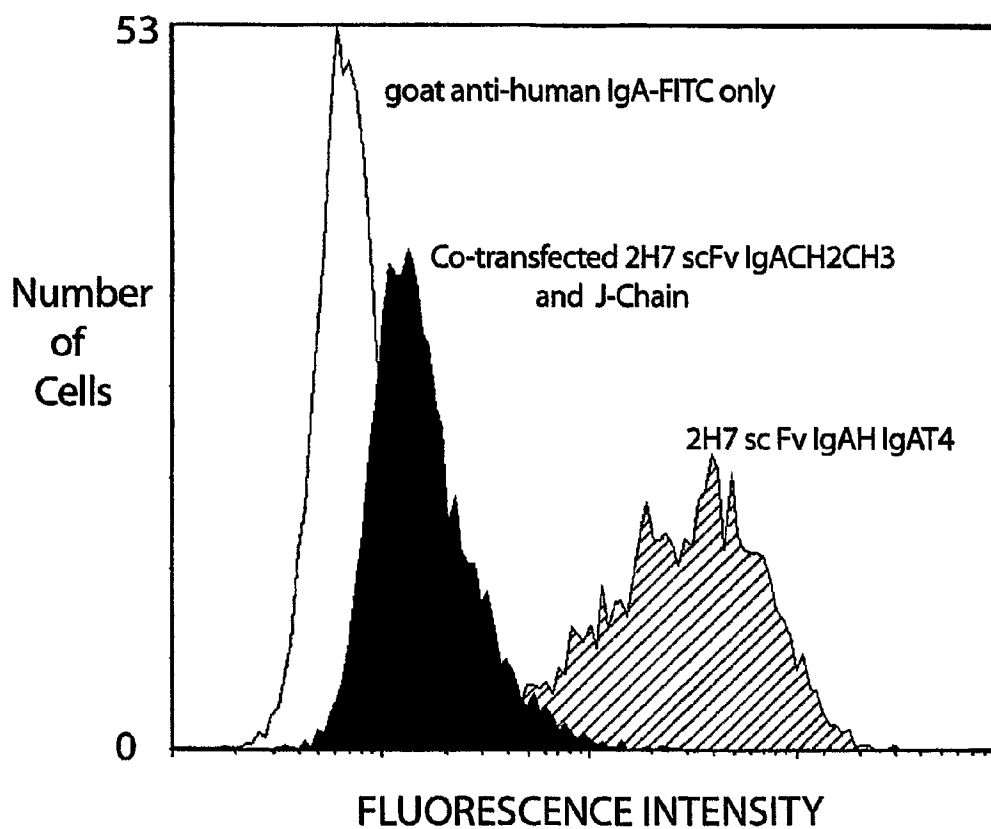


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FIG.36

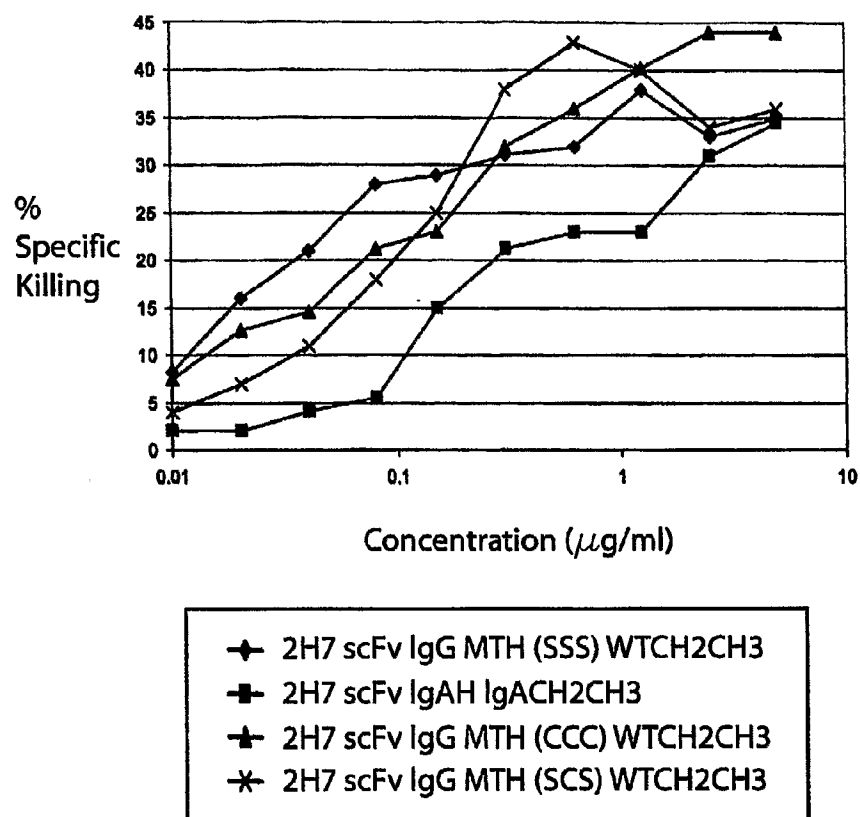


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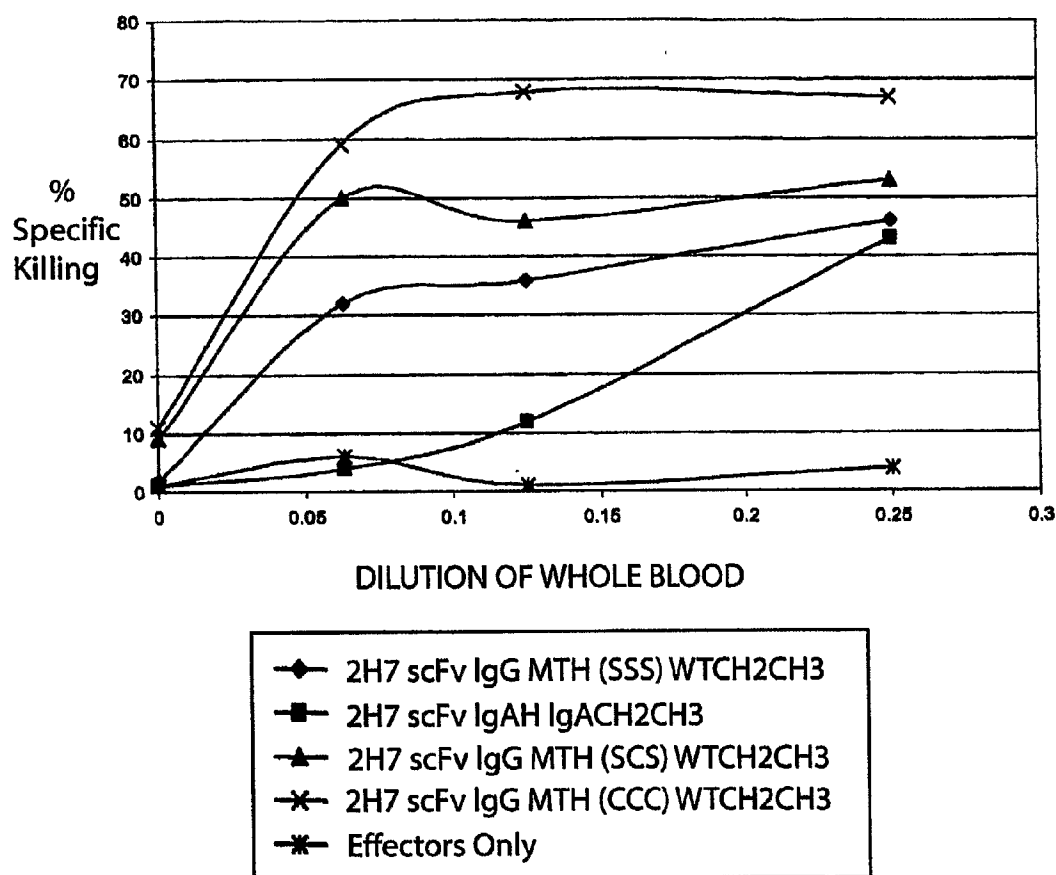
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FIG.37



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FIG.38



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FIG.39A

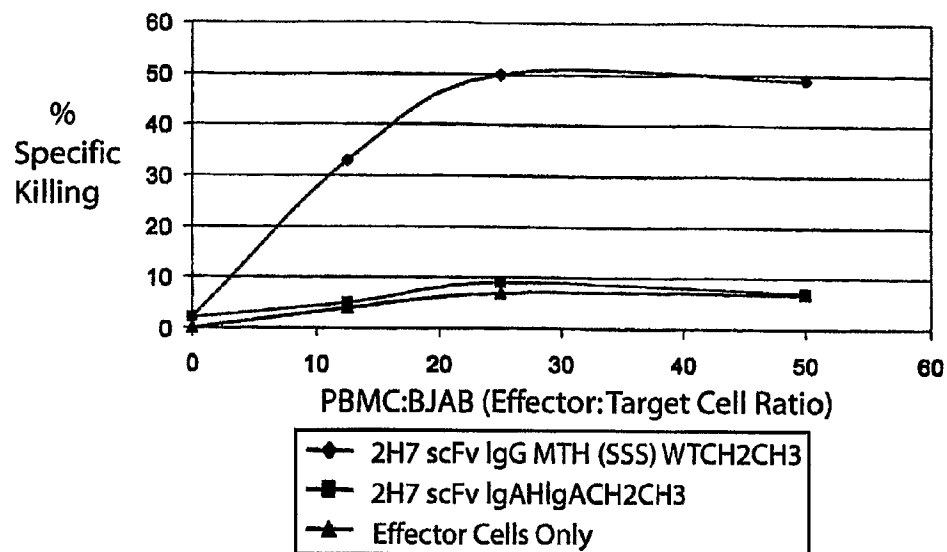
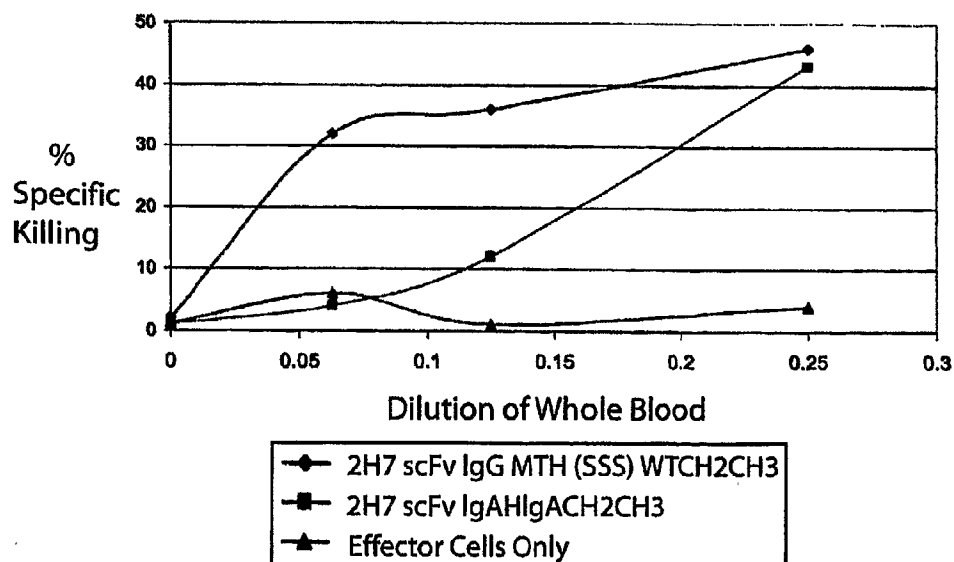


FIG.39B



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FIG.40

